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MICROBIC VIRULENCE AND HOST SUSCEPTIBILITY IN PARATYPHOID-ENTERITIDIS INFECTION OF WHITE MICE.

VIII. The Effect of Selective Breeding on Host Resistance. Further Studies.

By LESLIE T. WEBSTER, M.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 24, 1925.)

In a previous paper[1] we reported a series of experiments relating to the effect of selective breeding on the resistance of mice to mouse typhoid infection and mercury bichloride poisoning. This work has now progressed to a point where further studies may be described, and a general summary stated.

All technique has been similar to that employed before.[1] Mouse typhoid bacilli, the epidemic M. T. II strain of *Bacillus pestis caviæ*, were administered to mice intrastomachally in known quantities, by means of a silver catheter and syringe. The Rockefeller Institute breed of mice, whose history and maintenance have been described,[2] was used throughout.

The published experiments relating to this study[1] may be reviewed as follows: At the outset, 100 Rockefeller Institute mice were inoculated *per os* with a given dose of mouse typhoid bacilli. 2 months later, ten survivors, males and females, which showed no mouse typhoid bacilli in blood or feces, and no specific agglutinins, were bred in the stock mouse room. Later, when fourteen of the resulting F_1 generation had reached a weight of 16 to 18 gm. each, they were inoculated in the same manner, together with twenty unselected mice of the same original strain as controls. 28 per cent of these survived, and 48 per cent of the F_1 selected mice. Subsequently, six of the F_1 survivors, free of mouse typhoid bacilli, were bred as before and 3 months later, twenty-one F_2 offspring and twenty-one controls were inoculated. 30 per cent of the controls and 85 per cent of the F_2 mice survived. This repeated selection and breeding of the resistant individuals of each generation

[1] Webster, L. T., *J. Exp. Med.*, 1924, xxxix, 879.

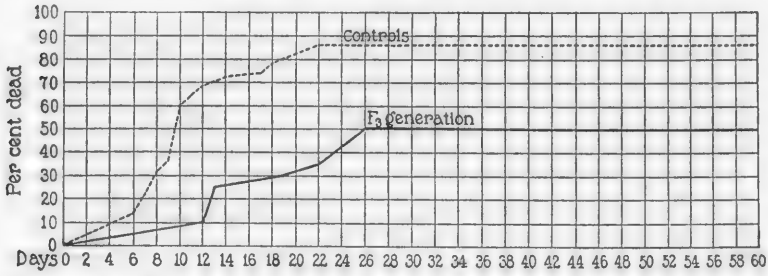
[2] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 195.

had apparently given rise to a strain of mice considerably more refractory to infection than the parent stock.

Since the publication of the above experiments, this procedure has been continued for three more generations.

Experiment 1.—Ten survivors of the F_2 generation, free of mouse typhoid bacilli in blood and stools, were bred in the mouse stock room. On May 9, 1924, twenty-one of the F_3 offspring, together with a control group of 50 mice, [2,3] were injected *per os* with the routine dose of mouse typhoid bacilli. Dead mice were autopsied and cultured as in the previous work.

The mortality of the F_3 generation and of the Rockefeller Institute controls is recorded in Text-fig. 1.[4] The death rate and final mortality



TEXT-FIG. 1. The susceptibility of the "resistant strain," fourth generation, to mouse typhoid infection.

of the selected mice were again much lower than those of the control group. But in addition to this, another fact was brought out by the experiment; namely, that influences which modify, in a seasonal manner, the susceptibility of different races of mice to mouse typhoid infection,[4] affect this group also. For, just as the mortality of five other races of mice injected at this time was higher than at any other period during the year, so the resistant group succumbed in larger numbers than before.

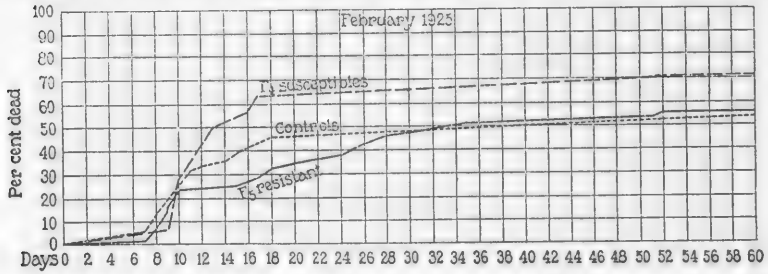
Only three survivors of the F_3 group were free of mouse typhoid bacilli in feces and specific blood agglutinins. These, two females and one male, were mated in the breeding room. 12 weeks later, fifteen F_4 offspring were available, but instead

[3] Pritchett, I. W., *J. Exp. Med.*, 1925, xli, 209.

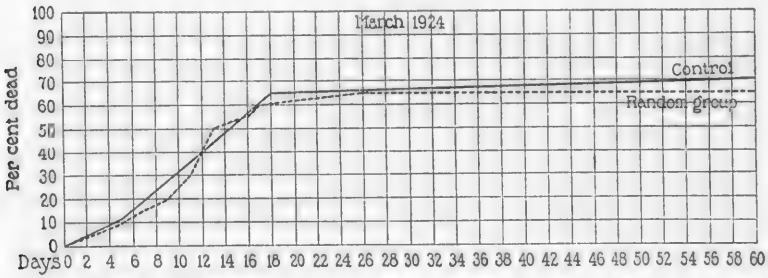
[4] Pritchett[3] (see Text-figs. 1 and 2).

of being inoculated as before, they were mated, brother to sister. On February 5, 1925, 42 of the sixth generation, F_5, together with a routine monthly series of 50 Rockefeller Institute controls, 50 Bagg, 50 Lathrop, and 50 Little mice (dilute brown), and also a group of so called "susceptible mice," which will be described later, were inoculated with the routine dose of mouse typhoid culture.

At this time, unfortunately, it was noticed that many of the F_5 mice were puny, underweight, cold, and sickly. No explanation was



TEXT-FIG. 2. The susceptibility of the "resistant strain," sixth generation, and of the "susceptible strain," fifth generation, to mouse typhoid infection.

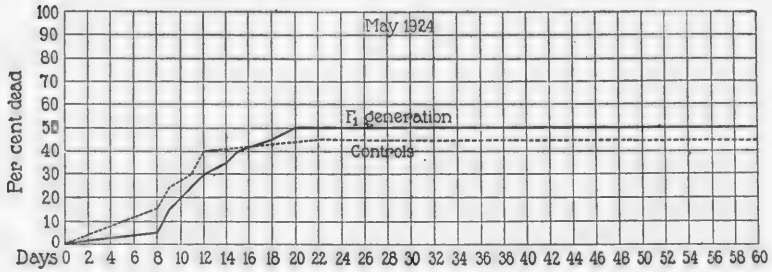


TEXT-FIG. 3. The susceptibility of a random group of the Rockefeller Institute strain to mouse typhoid infection.

at hand, so the group was used. That some disturbing element had appeared was evidenced by the mortality rate thereafter. The unhealthy mice succumbed quickly, and in numbers sufficient to bring the total mortality slightly higher than that of the unselected controls (Text-fig. 2). The mortality was more gradual, however, and would have been quite as usual if the weaklings dying from the 7th to the 10th days had been discarded.

While this series of experiments on the selective breeding of resistant mice was in progress, another strain was developed from the same original Rockefeller Institute stock by the repeated selection and breeding of the most susceptible individuals from a random group.

Experiment 2.—On March 5, 1924, twenty-one female mice, with litters 10 to 12 weeks old, were taken at random from the Rockefeller Institute stock, and numbered consecutively. The young mice were left in their respective cages, while the mothers were each placed in a separate jar with marks of identification, and taken to the experimental room. Each then received *per os* the usual dose of about 4,000,000 mouse typhoid bacilli in ½ cc. volume of broth.



TEXT-FIG. 4. The susceptibility of the "susceptible strain," second generation, to mouse typhoid infection.

The mortality of this group is shown in Text-fig. 3. The curve corresponds closely to the standard control curve for this race of mice.[2-5] Deaths were noted on the 5th day and continued quite regularly until the 18th day, after which only one fatality was recorded.

The litters of the most susceptible mothers, that is the first six mice to die, were selected, and twenty of these females were mated with four of the males. The F_2 offspring were old enough to be weaned on May 22, 1924. The cages and F_1 mothers were then numbered, and the latter were placed in separate jars and injected as before, together with a random group of twenty Rockefeller Institute controls.

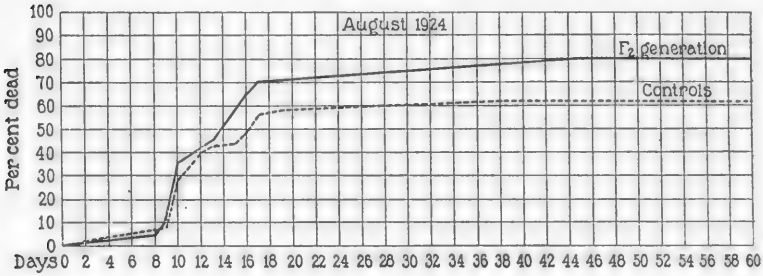
Both series reacted quite similarly to the mouse typhoid bacilli (Text-fig. 4). The mortality of the selected mice was slightly

[5] Webster, L. T., *J. Exp. Med.*, 1923, xxxvii, 231.

greater than that of the control group, but the differences could not be considered especially significant.

A second selection was made. The female offspring of the first six F_1 mothers to die were mated with four males from the first two of these litters. On August 15 the F_3 offspring were weaned and the twenty F_2 mothers were numbered accordingly, and taken to the experimental room for inoculation. All conditions and injection technique were kept uniform. 50 unselected Rockefeller Institute mice were inoculated as controls at the same time.

Text-fig. 5 compares the mortality of the two series. After the 8th day deaths were recorded frequently in both groups. On the 16th day 48 per cent of the controls and 65 per cent of the susceptibles were dead, and after 2 months the final mortality of the



TEXT-FIG. 5. The susceptibility of the "susceptible strain," third generation, to mouse typhoid infection.

controls was 62 per cent, as compared to 80 per cent of the F_2 selected mice.

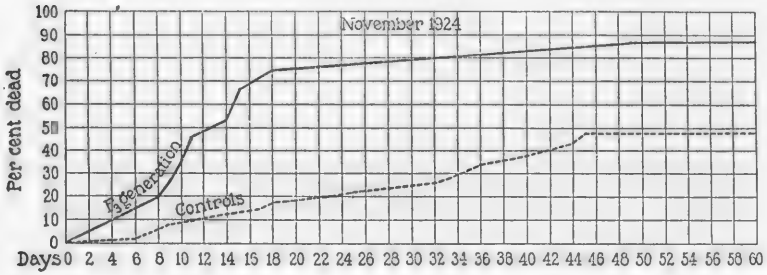
For a third time the litters of the most susceptible mothers were inbred and, on November 14, when the offspring had been weaned, the F_3 mothers, together with 50 Rockefeller Institute controls, and 50 of each of three other races, were given the usual dose of mouse typhoid bacilli.

The death rate of the selected mice was high and the final mortality 85 per cent (Text-fig. 6). The mortality of the control group (48 per cent), as well as that of the Bagg, Lathrop, and Little mice (dilute brown) strains, was very low.[6]

[6] Further details relating to the control curves in this series will be published by Dr. I. W. Pritchett.

A selection of offspring of the most susceptible individuals of the F_3 group was again made, and another breeding experiment carried out. On February 14, 1925, fourteen F_4 mothers were taken to the experimental room and, together with the routine monthly series of 150 mice, and the "resistant group" described above, were inoculated with the routine dose of mouse typhoid bacilli.

The mortality rates and figures of the "resistant," "susceptible," and "control" groups are plotted in Text-fig. 2, from which it may be seen that the susceptible mice died more rapidly and in larger numbers than either of the other series.



TEXT-FIG. 6. The susceptibility of the "susceptible strain," fourth generation, to mouse typhoid infection.

DISCUSSION.

The natural resistance of mice to pathogenic bacteria has been a subject of our studies for some time. In the first place, we have found that this quality of resistance is present in different amounts in individuals of the same family or race, and that these differences, under properly controlled conditions, take the form of a frequency curve. Furthermore, by an artificial selection of especially resistant or susceptible individuals it has been possible to breed strains at will, whose average resistance is greater or less than that of the original random group. It seems probable, then, that successive descendants of two given individuals inherit definite amounts of potential resistance, which vary according to the law of probability about a mean which approximates the mean resistance of the original pair.

It would seem that racial differences in resistance to mouse typhoid infection can be expressed by a relatively constant value.

Finally, whatever the potential inherited constitution of individuals, families, or races may be, it is affected profoundly by seasonal influences, food, and general hygienic conditions. For this reason it is erroneous to speak of the presence or absence of resistance as though it were a unit factor. Rather it should be considered a manifestation of an extremely complex mechanism, modified by heredity and environment, whose quantity in individuals of a large group tends to follow the laws of chance, about a mean which is more or less a characteristic of the race.

CONCLUSIONS.

1. Survivors of an experimental mouse typhoid infection, selected and bred for a number of consecutive generations, give birth to offspring which are more resistant to the disease than a random group of the same inbred race.

2. Offspring of females most susceptible to this infection give birth to individuals which are more susceptible than a group of similar unselected mice.

SURFACE TENSION OF SERUM.

XIV. Concerning the Change in Surface Tension Occurring as a Result of Immunization.

By P. LECOMTE du NOÜY, Sc.D., and LILLIAN E. BAKER, Ph.D.

(*From the Laboratories of The Rockefeller Institute for Medical Research.*)

(Received for publication, April 23, 1925.)

A considerable increase in the value of the time-drop in the surface tension of serum occurs as the result of immunization.[1,2] This phenomenon is obviously not due to the antibody itself since it reaches a maximum on the 13th day after an immunizing injection and disappears long before the disappearance of the antibody; and yet it appears to be associated with antibody formation since the two occur simultaneously. In order to ascertain whether this physicochemical change in immune serum is connected with antibody production or with other phenomena consequent on the antigen injection, two series of experiments were undertaken.

It has been reported[3] that in some cases an increase in the per cent of globulin and a decrease in the per cent of albumin is observable in immune sera, and it was thought that the increase in the time-drop might be traced to this change.

Seven rabbits, the sera of which showed a normal time-drop, were chosen for the experiments. Two were kept as controls and the others injected, after bleeding, with antigens as follows: one with Landsteiner antigen (horse kidney),[1] two with sheep cells, one with *Bacillus coli* culture, and one with crystalline egg albumin. The sera taken before injection, and also that taken on the 13th day after immunization, were analyzed for the per cent of globulin and albumin, sodium

[1] du Noüy, P. L., *J. Exp. Med.*, 1925 , xli, 779.

[2] du Noüy, P. L., *J. Exp. Med.*, 1922, xxxv, 575, 707; xxxvi, 547; 1923, xxxvii, 659; 1924, xl, 129.

[3] Doerr, R., and Berger, W., *Z. Hyg. u. Infectionskrankh.*, 1921, xciii, 147. Berger, W., *Z. ges. exp. Med.*, 1922, xxviii, 1.

TAB-

	Before antigen injection.										
	Surface tension.									Hemolysis.	
	Feb. 10, 1925.			Feb. 16, 1925.			Globulin.	Albumin in total protein.	Serum.	Hemolysis.*
	Dilution.	Initial value.	After 2 hrs.	Time-drop.	Initial value.	After 2 hrs.	Time-drop.				
		dynes	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	%	%	%	%
Rabbit 9. Landsteiner's antigen. 10 cc. injected Feb. 18, 1925.	1/8000	75.4	67.0	8.4	76.0	66.5	9.5	31	69	90	
	1/9000	75.4	66.9	8.5	75.8	67.0	8.8			80	
	1/9500	75.4	67.1	8.3	75.9	67.2	8.7	Ratio = 2.22		70	40
	1/10000	75.3	67.8	7.5	75.8	67.8	8.0			50	30
	1/10500	75.3	68.0	7.3	76.0	67.0	9.0			40	
	1/11000	75.4	68.3	7.1	76.0	68.0	8.0			30	20
	1/11500	75.6	68.4	7.2	76.0	68.5	7.5			20	15
	1/12000	75.5	68.7	6.8	76.0	69.0	7.0				
Rabbit 10. Sheep cells. 10 cc. 10% suspension injected Feb. 18, 1925.	1/8500	75.5	65.4	10.1	76.0	65.5	10.5	29	71	90	100
	1/9000	75.8	66.0	9.8	75.8	66.1	9.7			80	70
	1/9500	75.7	66.3	9.4	76.0	66.0	10.0	Ratio = 2.45		70	50
	1/10000	75.6	66.8	8.8	76.0	66.0	10.0			50	30
	1/10500	75.4	67.0	8.4	76.0	67.0	9.0			40	
	1/11000	75.4	65.8	9.6	76.0	67.3	8.7			30	20
	1/11500	75.4	66.0	9.4	75.6	67.5	8.1			20	10
	1/12000	75.4	66.8	8.6	75.8	68.0	7.8				
Rabbit 11. Sheep cells. 10 cc. 10% suspension injected Feb. 18, 1925.	1/8500	75.4	65.4	10.0	75.0	65.0	10.0	22	78	90	50
	1/9000	75.3	65.2	10.1	75.0	65.0	10.0			80	40
	1/9500	75.4	65.2	10.2	76.0	65.2	10.8	Ratio = 3.5		70	30
	1/10000	75.7	65.4	10.3	76.0	65.5	10.5			50	30
	1/10500	75.3	65.0	10.3	76.0	65.5	10.5			40	25
	1/11000	75.3	66.5	8.8	75.9	66.0	9.9			30	20
	1/11500	75.3	66.1	9.2	76.1	66.0	10.1			20	15
	1/12000	75.3	66.8	8.5	76.0	67.3	8.7				
Rabbit 12. Egg albumin, crystalline. 10 cc. 7.73% solution injected Feb. 18, 1925.	1/8500	75.7	68.4	7.3	74.2	65.0	9.2	29	71	0.25	0.25 (10^{-1})	—
	1/9000	75.7	67.3	8.4	74.2	65.0	9.2					
	1/9500	75.7	68.1	7.6	74.2	65.0	9.2	Ratio = 2.45		0.25	0.25 (10^{-2})	—
	1/10000	75.6	67.2	8.4	74.2	65.0	9.2					
	1/10500	76.0	68.4	7.6	74.0	65.0	9.0			0.25	0.25 (1/500)	—
	1/11000	75.3	67.8	7.5	74.2	65.8	8.4					
	1/11500	75.2	68.7	6.5	74.2	66.0	8.2			0.25	0.25 (1/1000)	—
	1/12000	75.3	67.8	7.5	74.2	66.0	8.2			0.25	0.25 (1/2000)	—

	Precipitin.		
	Serum.	E. alb. 7.7%.	Ppt.†
	cc.	*cc.*	
Rabbit 12. Egg albumin, crystalline. 10 cc. 7.73% solution injected Feb. 18, 1925.	0.25	0.25 (10^{-1})	—
	0.25	0.25 (10^{-2})	—
	0.25	0.25 (1/500)	—
	0.25	0.25 (1/1000)	—
	0.25	0.25 (1/2000)	—

* The hemolysis test was made with seven different concentrations of sera in 0.9 per cen volume, incubating for 1 hour at 37° and standing overnight in the ice box. The per cent of and distilled water.

† The precipitin reaction was carried out with crystallized egg albumin (7.73 per cent) at fiv

‡ ++ indicates complete; +, incomplete; ±, slight.

I.

13 days after antigen injection.									Sera treated with antigen *in vitro*.								
Surface tension.					Hemolysis.				Surface tension.								
Mar. 2, 1925.			Globulin.	Albumin in total protein.	Serum.	Hemolysis.			Before addition of antigen.			After 13 days incubation.					
												Before stirring.			After stirring.		
ial ue.	After 2 hrs.	Time-drop.							Initial value.	After 2 hrs.	Time-drop.	Initial value.	After 2 hrs.	Time-drop.	Initial value.	After 2 hrs.	Time-drop.
nes	*dynes*	*dynes*	%	%	%	%			*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*
5.5	63.0	12.5	29	71	30	100			75.7	66.0	9.7	75.0	67.0	8.0	74.6	64.9	9.7
5.5	63.0	12.5			20	90			75.7	66.3	9.4	75.1	66.8	8.3	74.6	66.9	9.7
5.6	63.7	11.9	Ratio = 2.45		10	30			75.7	66.5	9.2	75.1	66.8	8.3	74.5	67.0	7.5
5.5	63.0	12.5			9	20			75.7	65.8	9.9	75.0	67.6	7.4	74.5	67.2	7.3
5.4	62.8	12.6			8	15			75.7	75.0	0.7	74.9	67.9	7.0	74.6	68.7	5.9
5.5	61.9	13.6			7	0			75.6	68.0	7.6	75.0	67.6	7.4	74.5	64.6	9.9
5.6	61.8	13.8			6				75.8	68.5	7.3	74.8	68.5	6.3	74.5	66.9	7.6
5.6	63.6	12.0			5				76.0	70.0	6.0	74.7	68.0	6.7	74.5	67.0	7.5
					4												
5.5	64.0	11.5	31	69	30	100			74.5	63.1	11.4	75.0	67.7	7.3	74.8	68.7	6.1
5.4	64.7	10.7			20	100			74.5	64.0	10.5	74.9	67.4	7.5	74.8	68.9	5.9
5.4	64.8	10.6	Ratio = 2.25		10	100			75.0	64.6	10.4	74.9	67.8	7.1	74.7	68.0	6.7
5.4	65.8	9.6			9	100			74.5	65.3	9.2	74.9	67.8	7.1	74.7	69.0	5.7
5.5	64.5	11.0			8	100			74.6	65.4	9.2	74.9	68.8	6.1	74.9	69.2	5.7
5.4	64.9	10.5			7	100			74.5	64.7	9.8	74.9	69.3	5.6	74.8	69.9	4.8
5.5	65.7	9.8			6	100			74.5	66.0	8.5	74.9	69.0	5.9	74.8	67.2	7.6
5.5	67.0	8.5			5	100			74.7	66.7	8.0	74.9	69.5	5.4	74.8	68.0	6.8
					4	80											
5.5	63.0	12.5	26	74	30	100			75.0	63.5	11.5	74.9	67.0	7.9	74.9	67.4	7.5
5.5	63.0	12.5			20	100			75.0	64.0	11.0	74.9	67.1	7.8	75.0	68.0	7.0
5.4	62.9	12.5	Ratio = 2.85		10	100			75.0	64.0	11.0	74.9	67.9	7.0	75.0	69.0	6.0
5.4	60.7	14.7			9	100			75.0	64.9	10.1	75.0	68.7	6.3	75.3	68.6	6.7
5.4	63.0	12.4			8	100			75.4	64.4	11.0	74.9	69.0	5.9	74.6	67.6	7.0
5.6	63.8	11.8			7	100			75.0	65.1	9.9	75.0	69.0	6.0	74.7	66.6	8.1
5.5	63.9	11.6			6	80			75.1	65.5	9.6	75.0	69.0	6.0	74.8	68.0	6.8
5.5	64.0	11.5			5	80			75.1	65.3	9.8	75.0	69.3	5.7	74.8	71.0	3.8
					4	70											
					Precipitin.												
					Serum.	E. alb.	Ppt.										
					cc.	*cc.*											
75.5	63.5	12.0	27	73	0.5	0.5 ($\frac{1}{10}$)	++		75.6	63.9	11.7	75.3	65.0	10.3	74.7	66.5	8.2
75.5	63.3	12.2							75.4	64.6	10.8	75.3	65.4	9.9	74.6	66.4	8.2
75.4	63.3	12.1	Ratio = 2.70		0.5	0.5 ($\frac{1}{100}$)	±		75.4	65.0	10.4	75.3	64.9	10.4	74.6	66.5	8.1
75.4	63.0	12.4							75.4	65.8	9.6	75.3	65.7	9.6	74.7	67.6	7.1
75.5	63.0	12.5			0.5	0.5 ($\frac{1}{500}$)	—		75.4	66.0	9.4	75.3	66.2	9.1	74.4	66.2	8.2
75.5	64.8	10.7							75.4	66.0	9.4	75.0	66.9	8.1	74.5	66.2	8.3
75.5	63.9	11.6			0.5	0.5 ($\frac{1}{1000}$)	—		75.7	66.0	9.7	75.3	66.6	8.7	74.6	68.7	5.9
75.5	66.5	9.0							75.6	65.7	9.9	75.0	66.0	9.0	74.6	65.9	8.7
					0.5	0.5 ($\frac{1}{5000}$)	—										

alt solution, on sheep cells washed six times with 0.9 per cent NaCl and made up to original emolysis was determined by comparison with a set of standards made from the same sheep cells

ilutions from 1 in 10 parts to 1 in 5,000 parts.

TABLE I

	Before antigen injection.												
	Surface tension.												
	Feb. 10, 1925.				Feb. 16, 1925.			Globulin.	Albumin in total protein.	Serum.	NaCl.	*B. coli.*	Agg.§
	Dilution.	Initial value.	After 2 hrs.	Time-drop.	Initial value.	After 2 hrs.	Time-drop.						
		dynes	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	%	%		*cc.*	*cc.*	
Rabbit 13. *B. coli.* 10 cc. 24 hr. culture injected Feb. 18, 1925.	$\frac{1}{8}$	75.7	68.4	7.3	74.2	65.0	9.2	24	76	(*a*) 1 cc. ($\frac{1}{4}$)	1	1	–
	$\frac{1}{9}$	75.7	67.3	8.4	74.2	65.0	9.2						
	$\frac{1}{10}$	75.7	68.1	7.6	74.2	65.0	9.2	Ratio = 3.16		(*b*) 1 " (*a*)	1	1	–
	$\frac{1}{10}$	75.6	67.2	8.4	74.2	65.0	9.2						
	$\frac{1}{10}$	76.0	68.4	7.6	74.0	65.0	9.0			(*c*) 1 " (*b*)	1	1	–
	$\frac{1}{11}$	75.3	67.8	7.5	74.2	65.8	8.4						
	$\frac{1}{11}$	75.2	68.7	6.5	74.2	66.0	8.2			(*d*) 1 " (*c*)	1	1	–
	$\frac{1}{12}$	75.3	67.8	7.5	74.2	66.0	8.2						

	Hemolysis.	
	Serum.	Hemolysis.*
	%	%
Rabbit 14. Control. 10 cc. 0.9% solution NaCl Feb. 18, 1925.	50	–
	30	–
	20	–
	10	–
	7	–
	6	–
	5	–
	4	–
Rabbit 15. Control.	50	10
	30	5
	20	–
	10	–
	9	–
	8	
	7	
	6	
	5	

	Rabbit 14.												
	$\frac{1}{8}$	74.4	64.1	10.3	74.5	67.4	7.1	25	75				
	$\frac{1}{9}$	74.4	64.3	10.1	74.5	67.8	6.7						
	$\frac{1}{10}$	74.5	64.9	9.6	74.6	67.3	7.3	Ratio = 3.00					
	$\frac{1}{10}$	74.4	65.2	9.2	74.8	69.0	5.8						
	$\frac{1}{10}$	74.6	62.8	11.8	74.8	68.7	6.1						
	$\frac{1}{11}$	74.5	65.3	9.2	74.8	68.0	6.8						
	$\frac{1}{11}$	74.6	71.2	3.4	74.8	68.8	6.0						
	$\frac{1}{12}$	74.9	65.9	9.0	74.8	69.5	5.3						
	Rabbit 15.												
	$\frac{1}{8}$	74.8	63.5	11.3	74.9	64.3	10.6	22	78				
	$\frac{1}{9}$	74.3	64.1	10.2	74.9	64.8	10.1						
	$\frac{1}{10}$	74.4	64.0	10.4	74.6	66.4	8.2	Ratio = 3.50					
	$\frac{1}{10}$	74.4	64.0	10.4	74.6	65.8	8.8						
	$\frac{1}{10}$	74.5	64.4	10.1	74.6	65.8	8.8						
	$\frac{1}{11}$	74.5	64.2	10.3	74.6	64.9	9.7						
	$\frac{1}{11}$	74.6	63.6	11.0	74.7	66.5	8.2						
	$\frac{1}{12}$	74.6	64.9	9.7	75.0	66.5	8.5						

Before immunization mean ratio of 4 experiments = 2.65

Mean ratio of 2 controls = 3.25

§ In the agglutination test 1 cc. 0.9 per cent NaCl was placed in each tube, 1 cc. of 1/4 a dense suspension of a 24 hour culture of *B. coli* was used in each.

Concluded.

13 days after antigen injection.							Sera treated with antigen *in vitro*.								
Surface tension.					Hemolysis.		Surface tension.								
Mar. 2, 1925.			Globulin.	Albumin in total protein.	Serum.	Hemolysis.	Before addition of antigen.			After 13 days incubation.					
										Before stirring.			After stirring.		
Initial ue.	After 2 hrs.	Time-drop.					Initial value.	After 2 hrs.	Time-drop.	Initial value.	After 2 hrs.	Time-drop.	Initial value.	After 2 hrs.	Time-drop.
nes	*dynes*	*dynes*	%	%	%	%	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*	*dynes*
							75.4	69.4	6.0	75.4	65.8	9.6	74.6	67.5	7.1
							75.4	65.7	9.7	75.4	66.4	9.0	74.6	66.8	7.8
							75.4	68.6	6.8	75.4	67.6	7.8	74.7	67.3	7.4
							75.4	69.0	6.4	75.5	66.8	8.7	74.7	67.6	7.1
							75.6	70.0	5.6	75.6	67.6	8.0	74.7	67.6	7.1
							75.7	69.7	6.0	75.5	66.4	9.1	74.7	68.4	6.3
							75.5	70.1	5.4	75.5	67.7	7.8	74.7	68.5	6.2
							75.4	70.4	5.0	75.7	67.4	8.3	74.5	68.7	5.8
.7	65.0	9.7	30	70	50	—	75.9	68.7	7.2	75.5	64.0	11.5	74.6	62.8	11.8
.7	64.5	10.2			30	—	75.7	69.0	6.7	75.5	63.8	11.7	74.7	62.8	11.9
.6	65.5	9.1	Ratio = 2.35		20	—	75.4	68.2	7.2	75.5	65.8	9.7	74.6	62.2	12.4
.8	66.0	8.8			10		75.7	68.6	7.1	75.5	65.8	9.7	74.7	62.5	12.2
.6	66.5	8.1			7	—	75.7	68.8	6.9	75.4	64.9	10.5	74.6	64.9	9.7
.8	66.7	8.1			6	—	75.7	70.4	5.3	75.5	67.3	8.2	74.7	67.3	7.4
.7	66.0	8.7			5	—	75.8	69.8	6.0	75.5	67.3	8.2	74.7	67.2	7.5
.7	66.1	8.6			4	—	75.4	69.3	6.1	75.5	67.4	8.1	74.6	67.6	7.0
.7	63.7	10.0	23	77	50	10	75.6	66.4	9.2	75.3	60.8	14.5	74.5	62.5	12.0
4 8	65.0	9.8			30	5	75.6	66.0	9.6	75.3	61.4	13.9	74.6	63.8	10.8
.8	65.1	9.7	Ratio = 3.35		20	—	75.6	67.1	8.5	75.4	61.9	13.5	74.5	63.8	10.7
.9	63.9	10.0			10	—	75.6	66.5	9.1	75.4	63.7	11.7	74.5	64.2	10.3
.8	65.2	9.6			9	—	75.4	67.9	7.5	75.4	63.9	11.5	74.6	64.4	10.2
.7	66.0	8.7			8	—	75.3	68.3	7.0	75.4	64.8	10.6	74.6	64.4	10.2
.7	66.0	8.7			7		75.5	68.5	7.0	75.3	65.9	9.4	74.6	61.9	12.7
.8	66.6	8.2			6		75.6	68.8	6.8	75.3	65.4	9.9	74.6	62.3	12.3
			After immunization mean ratio of 4 experiments = 2.56												
			Mean ratio of 2 controls = 2.82												

erum was added to the first and mixed, then 1 cc. of this carried to the next tube, etc. 1 cc. of

sulfate being used for the precipitation of the globulin and the per cent of nitrogen being determined by the micro Kjeldahl method before and after precipitation.

A slight variation in the usual technique of precipitating the globulins was made to avoid the difficulty of bumping, so often experienced in the micro Kjeldahl method because of the high concentration of sodium sulfate. 1 cc. of serum was diluted with 2 cc. of 0.9 per cent NaCl solution. To this was added 6 cc. of 33 per cent sodium sulfate, which produced a final concentration of 22 per cent sodium sulfate and diluted the serum 1 to 9. This mixture was allowed to stand for 3 hours at 37°C., then filtered through double hardened filters, refiltered until clear, and 2 cc. samples of the filtrate were taken for the Kjeldahl determination. This 2 cc. contained just enough sodium sulfate for a Kjeldahl and enough protein for a micro determination.

As is seen from Table I, no constant change of any magnitude occurred in the per cent of albumin or globulin as the result of immunization with any of the antigens. The magnitude of the time-drop of all the sera was taken on the 13th day, according to the technique of du Noüy,[4] and the usual increase was found to have taken place in the sera of all the immunized animals, whether the albumin-globulin ratio remained identical with the original ratio, or changed very slightly. It is evident, therefore, that the increase in the time-drop of serum on immunization cannot be traced to an altered albumin-globulin ratio.

The same set of animals was used for the second experiment, to determine whether the antigen itself, or a substance produced by its direct action on some constituent of the serum is responsible for change in the time-drop. The question here involved is that of whether the change results from a reaction between the antigen and the serum *in vitro* or whether the presence of living tissue cells is an essential condition for its production.

10 cc. of the serum of each animal, taken before immunization, was mixed in test-tubes with a *pro rata* quantity of the immunizing antigen. In calculating this quantity the assumption was made that the animal had 70 cc. of blood for each kilo of body weight and that of this blood 40 per cent was plasma. The same antigen was used with each serum that was employed in the later immunization of the animal. The

[4] du Noüy, P. L., *J. Gen. Physiol.*, 1923–24, vi, 625.

time-drop in surface tension was measured before and after the addition of the antigen. The tubes were then incubated under sterile conditions in a Freas constant temperature water bath at 37.5°C. for 13 days. At the end of that time, the time-drop was again measured. Two sets of measurements were taken, one before stirring the sera, and one after agitation, since a slight precipitate formed in some cases. The results are given in Table I. It is seen that there is no increase in the magnitude of the time-drop in any of these sera, although it occurred in all cases in which the animal was immunized with antigen.

From the foregoing, it may be concluded that the physicochemical change in the serum to which the increased time-drop is due cannot be the result of the direct action of antigen on any constituent of the serum. It seems probable that it is an indirect action brought about through interaction with the tissues of the living animal. As might have been expected, none of the sera treated with the antigens *in vitro* yielded a positive precipitin or hemolysin reaction, while all of those from the immunized animals gave positive tests for such antibodies.

CONCLUSION.

The nature of the relation between the time-drop and antibody formation cannot be stated as yet. It is certain that the physicochemical change in the serum, detected by means of surface tension measurements, is not due directly to the antibody itself. Neither is it due to the direct action of the antigen on the serum, or to a change in the albumin-globulin ratio in this latter. Rather does it appear like antibody formation itself to be due to a tissue activity. Further experiments are being carried out on this subject.

STUDIES IN EXPERIMENTAL SYPHILIS.

III. Further Observations on the Possibility of Cure of Syphilis in the Rabbit with Arsphenamine.

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As a result of his studies in apes experimentally infected with syphilis, Neisser (1) concluded that failure to reinoculate a syphilitic animal, whether treated or untreated, indicated the existence of the first infection, and that a successful reinoculation, on the other hand, indicated that the first infection had been eradicated. He, therefore, considered the reinoculation test as a valid criterion of cure in experimental syphilis in apes. His view has been accepted by Kolle (2) and others, although Uhlenhuth and Mulzer (3) are doubtful if the method may be applied to rabbits.

In a previous communication (4) we reported experiments which cast some doubt upon the validity of the reinoculation test in rabbits as an index of cure. Briefly, it was shown that syphilitic rabbits treated late in the course of the infection, at a time when all the signs of activity of the disease had disappeared, could with appropriate treatment be rendered sterile so far as their lymph nodes were concerned, but, nevertheless, were refractory to a second (intracutaneous) inoculation. If one were to judge by the behavior of the lymph nodes in these treated animals, they had been freed of the infection, whereas, if one were to use the reinoculation test as a criterion of cure, it would have to be assumed that treatment had been ineffectual in eradicating the infection. In order to reconcile these apparently discrepant results the explanation was advanced that perhaps the failure to reinoculate a treated syphilitic rabbit was due not to the presence of a still existing uncured infection but to the presence of a resistance which the animal had acquired as the result of the infection and which persisted after its abolition through treatment. It seemed

advisable to obtain more information upon this question, if possible, by determining the behavior toward reinoculation of syphilitic animals treated early in the course of the disease and that of animals treated late, together with a determination of the infectivity of the lymph nodes just prior to, and also a considerable time after reinoculation.

Kolle (2, 5) has carried out an extensive series of reinoculations in treated and untreated syphilitic rabbits. In brief, he found that if treatment is begun up to 45 days after the first inoculation, the animals are susceptible to a second infection (reinoculation). If treatment is begun between the 45th and the 90th days, reinoculation yields variable results, whereas, if it is not begun until after the 90th day, the animals are uniformly insusceptible to a second infection. Whether treatment was prolonged or not made no difference. He interpreted these results as indicating that cure of syphilis in the rabbit is regularly accomplished if treatment is begun before the 45th day after inoculation, is questionable if it be instituted as late as the period between the 45th and 90th days of the disease, and is impossible if it be postponed until after the 90th day. Similar results have been obtained by Frei (6).

As will be seen, our experiments parallel those of Kolle very closely in their results, particularly as regards the relationship between reinoculability and time at which antisyphilitic treatment is begun. However, they contain additional data which appear to contradict the interpretation advanced by him, and indicate that failure to reinoculate a treated syphilitic rabbit should perhaps more properly be regarded as an expression of resistance to infection and may not necessarily mean the persistence of the first infection.

EXPERIMENTAL.

Experiment 1.—This experiment comprised six groups of adult male rabbits of various breeds. Care was taken to distribute representatives of various breeds in the different groups. All groups were inoculated intratesticularly with the Nichols strain of *Treponema pallidum*. Four groups (A, C, D, E) were treated in exactly the same manner; namely, with six weekly intravenous injections of arsphenamine, the individual dose being 10 mg. per kilo.

Group A. Five Adult Males.—Treatment was begun 49 to 50 days after inoculation, or early in the course of the disease.

Group B. Four Adult Males.—Untreated controls.

Group C. Five Adult Males.—Treatment was begun 181 to 291 days after inoculation, or late in the course of the infection.

Group D. Three Adult Males.—Control on Group A, not reinoculated. Treatment was begun on the 49th day of the disease.

Group E. Three Adult Males.—Control on Group C, not reinoculated. Treatment was begun 181 to 226 days after inoculation.

Group F. Five Adult Males.—Virus control on reinoculations. Not treated.

Groups D and E each comprised more animals at the beginning of the experiment but several animals were lost through an intercurrent infection.

At least 49 days after the completion of treatment a single popliteal node was excised under ether anesthesia from each of the animals in Groups A, B, and C, emulsified in physiological saline solution, and injected intratesticularly into each of two normal rabbits. The entire amount of emulsion was utilized. Following this procedure all the animals in these three groups were reinoculated with an emulsion of syphilitic rabbit testis containing numerous actively motile treponemata. The minimum time interval between the end of treatment and reinoculation was 53 days. Previous experiments had shown that this time interval was more than sufficient to allow the effects of treatment to wear off.

Reinoculations were made in each of two ways: (*a*) by intracutaneous inoculation at the base of the ear, and (*b*) by inoculation of the opposite testicle from that originally infected. Five rabbits (Group F) were used to control the virus used for reinoculation. Of the four animals in the untreated group, three had developed metastatic orchitis prior to reinoculation, likewise of the four surviving animals in the late treated group, three had developed metastatic orchitis. At the time of reinoculation the metastatic orchitis had subsided. Reinoculations were, therefore, in six out of eight animals in these two groups, made in a testicle which had previously been the seat of a syphilitic inflammation. In the group treated early, no metastatic orchitis developed prior to reinoculation, so that reinoculations in this group were all made in testicles which gave no clinical evidence of ever having been the seat of syphilitic inflammation.

Following reinoculation all the animals were observed for a period of 90 days and at the end of that time the remaining popliteal node was excised under ether anesthesia and transferred to each of two normal animals in the manner described above. This was done in order to determine whether or not dissemination of the organisms had taken place and as a check upon the reinoculation itself, since it is conceivable that reinoculation might be effected without the appearance of any local lesion at the site of inoculation and could be recognized in such a case only by finding the organisms in some other part of the body. The results, as will be seen later, amply justified this precaution. Lymph node transfers were made at the same time in the case of some of the virus control animals (Group F), and in those which had been treated but not reinoculated (Groups D and E).

In judging of the success of reinoculation we have been guided by (1) the clinical lesions produced at the site of inoculation, and (2) the results of the second lymph node transfer in the *treated* animals

only. An animal was regarded as having been successfully reinoculated if a characteristic lesion developed at the site of inoculation in which treponemata could be demonstrated, or if, in the case of the treated animals, the second lymph node transfer carried out after reinoculation was positive, when the first lymph node transfer made prior to reinoculation was negative.

All animals to which lymph node transfers were made were observed for 90 days before being discarded as negative.

The results of the experiment are shown in Table I.

The data presented in Table I may be briefly summarized as follows:

Group A.—Treatment early in the course of the disease (49th day) was sufficient to abolish lymph node infection. Four of the five animals upon reinoculation showed characteristic clinical lesions containing treponemata at one or other of the sites of inoculation, and all of them showed popliteal node infection, indicating that there had been generalization of the infection in all. In the four animals in which characteristic clinical lesions developed at the site of inoculation after the usual incubation period, the lesions went through the ordinary clinical course as observed in normal animals, healing spontaneously in time, and in two of these animals metastatic lesions in respect of keratitis developed. In the remaining animal no macroscopic lesion developed in the testicle but a questionable lesion occurred in the skin of the ear. No treponemata could be demonstrated in this lesion. In accordance with the criteria of a successful reinoculation, as outlined above, we have regarded the incidence of successful reinoculation in this group as being 100 per cent.

Group B.—In this, the untreated group, the first popliteal node transfer was positive in each animal, indicating the persistence of the original infection. All of the animals in this group were refractory to the second inoculation, and three of the four showed infected popliteal nodes at the time of the second transfer, which was 360 to 420 days after inoculation.

Group C.—In this group of five animals treated late in the course of the disease, lymph node infection was abolished in all. Unfortunately one of the animals was lost through pneumonia, so that only four were available for reinoculation, but all these proved refractory to a second infection so far as the production of a characteristic clinical lesion at the site of inoculation was concerned. However, one of these animals showed the presence of lymph node infection which was probably the result of the second inoculation. We have regarded this as evidence of successful reinoculation and have, therefore, considered that the incidence of successful reinoculations in this group was 25 per cent.

Group D.—All of the animals in this group, which constituted a control on Group A, and were not reinoculated, gave negative lymph node transfers 275 days after treatment.

TABLE I.

Group.	Rabbit No.	1st node transfer.	Reinoculation.			2nd node transfer.	
			Ear.	Testicle.	Generalized lesions.		
A	1	Neg.	?	Neg.	None.	Pos.	Successful reinoculations 100 per cent.
Treated early (49–50 days after inoculation).	2	"	Pos.	?	"	"	
	3	"	Neg.	Pos.	Eye.	"	
	4	"	"	"	None.	"	
	5	"	"	"	Eye.	"	
B	6	Pos.	"	Neg.	None.	"	" " 0 " "
Untreated.	7	"	"	"	"	"	
	8	"	"	"	"	Neg.	
	9	"	"	"	"	Pos.	
C	10	Neg.	"	"	"	"	
Treated late (181–291 days after inoculation).	11	"	"	"	"	Neg.	" " 25 " "
	12	"	Died from pneumonia.			—	
	13	"	Neg.	Neg.	None.	Neg.	
	14	"	"	"	"	"	
D	15	Not done.	Not reinoculated.			"	
Treated early (49 days after inoculation).	16	" "	"	"		"	
	17	" "	"	"		"	
E	18	" "	"	"		"	
Treated late (181–226 days after inoculation).	19	" "	"	"		"	
	20	" "	"	"		"	
F	21	—	?	Pos.	None.	Pos.	
Virus controls (reinoculation).	22	—	Pos.	"	"	"	
	23	—	?	"	"	"	
	24	—	Pos.	"	"	Not done.	
	25	—	"	"	"	" "	

Group E.—All of the animals in this group, which constituted a control on Group C, and were not reinoculated, likewise gave negative lymph node transfers 143 days after treatment was ended.

Group F. Virus Controls.—In all of the animals in this group characteristic syphilitic orchitis developed in the usual manner and time. Lymph node transfer was carried out in three of the five animals 90 days after infection and proved to be positive in each instance.

Summary.—Rabbits treated with arsphenamine early in the course of the infection, that is to say 49 to 50 days after inoculation, were rendered free of popliteal lymph node infection and were found to be uniformly susceptible to a second infection. Rabbits treated late in the course of the disease, 181 to 291 days after inoculation, were rendered free of lymph node infection but were refractory to a second infection in 75 per cent of the cases. Untreated syphilitic rabbits were found to exhibit lymph node infection 265 to 420 days after inoculation and to be uniformly refractory to a second infection. In animals treated either early or late in the course of the infection but not reinoculated, lymph nodes were found to be sterile as late as 275 days after the last dose of arsphenamine.

The results obtained in this experiment seemed to justify repetition upon a larger scale and, accordingly, a similar experiment was carried out, with larger groups of animals. The protocol follows.

Experiment 2.—Five groups of animals of various breeds were used. They were all inoculated intratesticularly with an emulsion of syphilitic rabbit's testis. The strain used was the same throughout; namely, the Nichols strain.

Group A. Nine Adult Males.—Treatment was begun 41 days after inoculation.

Group B. Four Adult Males.—Untreated control.

Group C. Nine Adult Males.—Treatment was begun 193 days after inoculation.

Group D. Four Adult Males.—First reinoculation control.

Group E. Two Adult Males.—Second reinoculation control.

In this experiment, treatment controls were eliminated since the previous experiment had shown that sterility of the lymph nodes produced by treatment persists for the duration of the experiment. In addition, Wassermann reactions were carried out on the animals at frequent intervals. In all of the animals in Group A the Wassermann reaction was positive when treatment was begun and became negative before it was completed. In Groups B and C the Wassermann reaction was negative in each animal at the beginning of treatment.[1]

[1] The technique employed in performing the Wassermann reaction in this experiment will not be presented here owing to lack of space. In a forthcoming

Treatment was the same as in the preceding experiment; namely, six intravenous doses of arsphenamine at weekly intervals, the single dose being 10 mg. per kilo of body weight. At the end of 49 days after the last dose of arsphenamine a single popliteal lymph node was removed under ether anesthesia from each animal in Groups A, B, and C, emulsified in salt solution and the emulsion transferred to the testes of two normal rabbits. The entire amount of the emulsion was utilized. 69 days after treatment was ended the first series of reinoculations was made. In this series inoculations were made intracutaneously at the base of the ear and also in the uninoculated testicle, the virulence of the virus being controlled with four normal rabbits (Group D). Of the eight animals in the early treated group only one had developed metastatic orchitis by the time treatment was begun, so that in this group reinoculations were made into a previously infected testis in one instance out of seven. In the late treated group metastatic orchitis had developed in five out of nine animals and had subsided before the beginning of treatment. Thus, the reinoculations in this group were made into a previously infected testicle in five out of nine instances. In the untreated group metastatic orchitis had been present in half of the animals but had subsided prior to the second inoculation.

39 days after *reinoculation* such animals as had not shown any clinical signs of a successful "take" were inoculated a second time, this time in the uninoculated testicle only. This second reinoculation was carried out in order to give those animals that had not developed clinical signs of a second infection an additional opportunity to do so. The virus used in the second attempt at reinoculation was controlled by two normal rabbits (Group E). 90 days after the first reinoculation the remaining popliteal node was removed under ether anesthesia from all the animals subjected to reinoculation and from several of the virus control animals. It was transferred to two normal rabbits in the manner already described. All animals to which lymph node transfers were made were observed for a period of 90 days before discarding them as negative.

The results of this experiment are shown in Table II.

Table II shows that, as a result of treatment, lymph node infection was abolished in all of the animals in the early treated group. In this group the incidence of clinical lesions produced at the site of reinoculation was low, but all the animals developed positive Wassermann reactions and in seven out of eight, the second lymph node transfer was positive. We have, therefore, considered the incidence

paper in this series it will be given in full together with our experience with the test in rabbit syphilis. It will suffice to say at this point that under proper precautions the Wassermann reaction is almost never positive in normal rabbits, whereas it is a remarkably constant finding in early active untreated syphilis of the rabbit.

TABLE II.

Group.	Rabbit No.	1st node transfer.	Reinoculation.				2nd node transfer.	
			Ear.	Testicle.	Generalized lesions.	Wassermann reaction.		
A	26	Neg.	Neg.	Neg.	None.	++	Pos.	Successful reinoculations 87.5 per cent.
Treated early (41 days after inoculation).	27	"	"	Pos.	"	++++	"	
	28	"	"	"	"	++++	"	
	29	"	"	Neg.	"	++	"	
	30	"	"	" *	"	++++	"	
	31	"	"	"	"	++++	"	
	32	"	"	"	"	++++	"	
	33	"	"	"	"	++++	Neg.	
B	34	"	"	"	"	0	"	
Untreated.	35	Pos.	"	"	"	0	"	
	36	"	"	"	"	0	"	
	37	"	"	"	"	++++	Pos.	
C	38	Neg.	"	"	"	0	Neg.	Successful reinoculations 0 per cent.
Treated late (193 days after inoculation).	39	"	"	"	"	0	"	
	40	"	"	"	"	0	"	
	41	"	"	"	"	0	"	
	42	"	"	"	"	0	"	
	43	"	"	"	"	0	"	
	44	"	"	"	Keratitis.†	0	"	
	45	"	"	"	None.	0	"	
	46	"	"	"	"	0	"	

D	47	—	Neg.	Pos.	Bone.	++++	Pos.
Virus controls (1st re-	48	—	"	"	None.	++++	Neg.
inoculation).	49	—	"	"	"	++++	Pos.
	50	—	"	"	"	++++	"
E	51	—	—	"	"	++++	Not done.
Virus controls (2nd re-	52	—	—	"	"	++++	" "
inoculation).							

* No macroscopic lesion in testis but actively motile treponemata were recovered from it.
† Involved cornea inoculated in two normal animals with negative result.

of positive reinoculations in this group as seven out of eight, or 87.5 per cent. In Animal 33 a positive Wassermann reaction appeared subsequent to reinoculation but lymph node transfer was negative. Syphilis could, therefore, not be proved to be present in this animal, consequently reinoculation was regarded as negative.

In the untreated group lymph node infection was present in three out of four animals just prior to reinoculation. In none of these animals was there any clinical evidence of a successful reinoculation. The second node transfer was negative in three out of four of the animals in this group. In the one animal (No. 37) in which it was positive, a recurrent positive Wassermann reaction developed after reinoculation. In this animal the course of the original infection was a relatively stormy one. The early lesions were numerous and extensive and there was a marked tendency to the development of large generalized lesions. In fact, this animal exhibited at one time or another skin, bone, and corneal lesions and constituted an excellent example of malignant syphilis in the rabbit.

In the late treated group, lymph node infection was abolished by treatment in all nine animals. In none of these animals was there the remotest sign of syphilitic infection at the site of reinoculation. None of the animals developed a positive Wassermann reaction subsequent to reinoculation and in all of them the lymph node transfer after reinoculation was negative. In one animal, No. 44, keratitis appeared 99 days after reinoculation. The cause for this keratitis is not clear. The affected eye was enucleated under ether anesthesia, the inflamed cornea excised, minced with scissors, and emulsified in physiological salt solution. The emulsion was examined for *Treponema pallidum* with dark-field illumination with negative result. It was then inoculated into the testes of two normal rabbits. Neither of these animals showed signs of syphilitic orchitis within 90 days. There is no proof, therefore, that this keratitis was syphilitic in origin, although it may have been. Inasmuch as the proof was not forthcoming and second lymph node transfer was negative, we have considered that this animal was not successfully reinoculated. The incidence of successful reinoculations in the late treated group was, therefore, considered to be 0 per cent, notwithstanding the fact that reinoculations were made once in the skin at the base of the ear and twice in the uninoculated testicle.

In the virus controls syphilitic orchitis developed in all the animals in the usual manner, but no lesions appeared in the ear. All of the animals developed positive Wassermann reactions and subsequent lymph node transfer was positive in three of the four virus control animals in which it was carried out.

The results of these two analogous experiments have been combined and presented in Table III.

Table III shows that of thirteen rabbits treated early in the course of syphilitic infection, that is to say 41 to 50 days after inoculation, lymph node infection was abolished in all. In twelve of the thirteen, or 92.3 per cent, reinoculations were successful. Of the thirteen rabbits treated late in the course of the disease, 180 to 193 days after inoculation, lymph node infection was likewise abolished in

TABLE III.

Combined Results of Experiments 1 and 2.

Group.	No. of rabbits.	Node transfer.				Reinoculation.			
		Positive.	Per cent.	Negative.	Per cent.	Positive.	Per cent.	Negative.	Per cent.
Treated early.....	13	0	0	13	100	12	92.3	1	7.7
" late.....	13	0	0	13	100	1	7.7	12	92.3
Untreated.....	8	7	87.5	1	12.5	0	0	8	100

all, but reinoculations were successful in only one of the thirteen, or 7.7 per cent. In eight untreated animals lymph node infection was still present in seven, or 87.5 per cent, in one instance as late as 420 days after inoculation, and all of these animals were refractory to a second inoculation.

DISCUSSION.

The results of the foregoing experiments are clear-cut and confirm those previously reported by us. The behavior of the early and late treated rabbits toward reinoculation is in complete agreement with that observed by Kolle. The time at which treatment of syphilitic infection in the rabbit is inaugurated determines the behavior of the animal toward a second infection. If treatment is instituted early

in the course of the infection, that is before the animal has had an opportunity to bring about the subsidence of the primary lesion, such an animal is susceptible to a second inoculation. On the other hand, if treatment is postponed until the animal has reacted to the disease, has brought about subsidence of the primary phenomena, and is in the stage of latency, then such an animal is almost always refractory to a second inoculation. Kolle, following the lead of Neisser, has taken the ground that this refractory state indicates that the animal has not been rid of its first infection by treatment, and concludes that it is impossible to cure syphilis in the rabbit with arsphenamine if the treatment is postponed as late as 90 days after inoculation. On the other hand, if it is instituted within 45 days after inoculation, cure is uniformly effected since such animals are uniformly susceptible to a second infection. In other words, he regards the reinoculation test as a satisfactory method for determining whether or not cure has been effected.

The results of lymph node transfer in our experiments indicate that perhaps the first infection may be eliminated by arsphenamine therapy no matter when the treatment is administered. We have found that lymph nodes of syphilitic rabbits treated with arsphenamine are uniformly rendered incapable of transmitting the infection to normal animals, no matter whether the treatment is begun early or late in the course of the disease. In not a single instance have we encountered lymph node infection in rabbits after treatment with arsphenamine. In this respect our results are exactly parallel to those of Nichols and Walker (7).

In view of these results it seems proper again to raise the question whether or not the reinoculation method is a valid test of cure or failure to cure, and whether the infectivity of the lymph nodes is not perhaps a more valid criterion.

Neisser (1) laid the experimental basis for the view that the reinoculation test is a valid one for the determination of cure of syphilis. He reinoculated twenty-nine untreated syphilitic apes and did not obtain any evidence of syphilitic disease at the site of the second inoculation. The reinoculations were carried out at periods ranging from 27 to 645 days after the first inoculation. In some instances two and even three reinoculations were attempted in the same animal.

Nevertheless, all the animals were refractory to reinfection. In twenty-two of them syphilitic infection could be demonstrated by inoculating normal apes with internal organs. From this, Neisser properly concluded that the apparently immune animals were for the most part still infected. This fact, together with his observation that many syphilitic apes, treated with various antisyphilitic agents and assumed to be cured, were capable of being reinoculated, led him to conclude that there is no true immunity to syphilis and that the reinoculation test constitutes a valid criterion of cure. He assumed that the treated apes were freed of their syphilitic infection since organ transfer from many of those which were not reinoculated was negative. It should be noted that not a few of Neisser's animals were treated early in the course of the disease and that in no instance were reinoculation and organ transfer carried out with the same animal. Kolle has taken the same ground in interpreting the reinoculation test in syphilitic rabbits, without, so far as we have been able to ascertain, carrying out any observations to determine whether or not the internal organs of treated syphilitic rabbits are infectious.

It seems to us that the reinoculation method tells only one thing; namely, whether or not the animal is refractory to a second infection. It does not necessarily follow that because a rabbit is refractory to a second infection by treponemata, that the first infection must needs be present. It may or it may not be. Finger (8) has said essentially the same thing. It is entirely possible, as Neisser himself stated, that an animal might be immune to a second inoculation in the absence of the first infection, but he admitted that he had never seen any evidence of it. The results of our experiments may be interpreted as indicating the existence of such an immunity in the absence of infection, and we are inclined to regard them in that light.

It must be admitted, however, that the possibility exists that abolition of lymph node infection by treatment does not necessarily mean that the syphilitic infection has been entirely eliminated. The treponemata in the lymph nodes may have all been killed by the therapeutic agent and yet there may still be foci of infection in the body which have not been reached. That phase of the problem has been investigated by us and the results of that investigation appear in the subsequent paper in this series.

The decision as to whether or not it is possible to bring about a biological cure of syphilis in the rabbit will depend, of course, upon the choice of criteria of cure. It is perhaps too early as yet to draw any definite conclusions as to which criterion is the more valid. Our experiments, several times repeated, have shown that the two methods of determining cure of this infection yield discrepant results in the same animal. Judged by the results obtained with the reinoculation method one would have to conclude with Kolle, and Frei as well, that cure of experimental syphilis in the rabbit is impossible if the treatment is begun late in the course of the disease. On the other hand, judged by the results obtained from a study of lymph nodes of treated animals, it is possible to cure the experimental infection no matter if treatment is postponed until 90 days or more after inoculation. It is clear that one or the other of these two criteria must be faulty.

Our experiments would also indicate that in judging whether or not reinoculation has been successful in rabbits, attention must be directed to factors other than the occurrence of a local syphilitic lesion at the site of reinoculation. This point has already been stressed by Brown and Pearce (9). In both of our experiments there were observed, in the treated groups, animals in which no local lesion developed at the site of reinoculation. Subsequent lymph node transfer was, however, positive, and, furthermore, a positive Wassermann reaction developed after reinoculation, when previously it had been negative. To our minds this would indicate that it is possible, under certain conditions, to reinoculate a rabbit and produce a generalized second infection without producing any recognizable lesion at the portal of entry. It is well known that the same holds true for the first infection (10). We have, therefore, come to the conclusion that before regarding any reinoculation in a treated syphilitic rabbit as negative, the lymph nodes must be transferred to normal rabbits. This view necessarily entails the use of many more rabbits in carrying out such work, but, in our opinion, it cannot be dispensed with.

SUMMARY.

Syphilitic rabbits can be treated with arsphenamine in such a manner as to render the lymph nodes incapable of transmitting the

infection to normal rabbits. This can be accomplished if treatment is begun either early or comparatively late in the course of the disease. If treatment is begun early, the animals are almost uniformly susceptible to a second infection, whereas, if it is begun late, they are almost uniformly refractory to a second infection. It is suggested that this refractory state in rabbits may be explained by the existence of an acquired immunity which persists after the abolition of the disease, rather than to the persistence of the first infection.

It would appear that it is possible under certain conditions to reinoculate rabbits and produce generalized infection without producing any lesion at the portal of entry.

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STUDIES IN EXPERIMENTAL SYPHILIS.

IV. The Survival of Treponema pallidum in the Internal Organs of Treated and Untreated Rabbits.

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In the foregoing paper of this series (1) it was shown that when syphilitic rabbits are treated with arsphenamine their reaction toward a second inoculation of treponemata will depend upon the time at which treatment was instituted. If arsphenamine is administered early in the course of the infection the animals are almost always susceptible to a second infection, whereas, if treatment is postponed until a period when the primary manifestations have subsided, such animals are almost always refractory to a second infection. These results are in entire agreement with the work of Kolle (2) and Frei (3). It was further shown that in the same treated animals the lymph nodes are rendered incapable of transmitting the syphilitic infection to normal rabbits no matter at what time the treatment is begun.

The bearing of these facts upon the question of the biologic cure of experimental syphilis in the rabbit was discussed and it was pointed out that under the conditions of the experiments the two criteria of cure, namely the reinoculation test and the lymph node test, could not both be valid, since they gave absolutely opposite results in the same animal. In the discussion it was pointed out that the abolition of lymph node infection by arsphenamine administration did not necessarily mean biologic cure, since it was conceivable that animals so treated might continue to harbor the organisms in the internal organs, although the lymph nodes had been rendered incapable of transmitting the infection to normal rabbits.

It seemed of paramount importance to determine whether or not syphilitic lymph node infection in the rabbit is an index of the presence

of syphilitic infection in more remote parts of the body both in treated and in untreated rabbits. The present communication deals with our attempts to answer that question.

HISTORICAL.

The relationship of lymph node infection to infection of the internal organs in syphilitic animals has not, so far as we are aware, been the subject of extensive study by workers in this field. Neisser (4), working with apes, demonstrated that the spleen and bone marrow were infectious in slightly over half of his animals, in one instance as late as 283 days after inoculation, and in half of the animals in which one or the other of these organs was infectious, the blood was also infectious. In one animal in which the lymph nodes and testicles were transferred to normal animals 70 days after inoculation, the result was positive. Uhlenhuth and Mulzer (5), working with syphilitic rabbits, showed that the inguinal lymph nodes harbor the infection and that the blood, the liver, and a mixture of spleen and bone marrow may be infectious but are not uniformly so. Pearce and Brown (6) found that the lymph nodes in distant parts of the rabbit's body regularly harbor the microorganism. They succeeded in recovering the virus from popliteal lymph nodes of rabbits as late as 51 months after inoculation. Eberson (7) recovered treponemata from the testes of rabbits as late as 4½ months after inoculation. Plaut and Mulzer (8) have recently succeeded in recovering treponemata from the brain of a rabbit inoculated intratesticularly with what they regard as a neurotropic strain of *Treponema pallidum*. The organisms were recovered by inoculating normal rabbits with brain substance removed 59 days after the original inoculation. No parallel inoculations with blood were reported by them, a fact which raises some question as to whether or not the organisms were really in the cerebral tissue outside the circulating blood, since it is well known that there is apt to be a spirochetemia in the early period of syphilitic infection in the rabbit.

EXPERIMENTAL.

Two groups of adult male rabbits, of various breeds, comprising in all ten animals, were used. All the animals were inoculated in the right testicle with a virulent strain of *Treponema pallidum* (Nichols strain), and all developed syphilitic orchitis in the usual manner. One group, of four animals, was untreated, the other group of six animals was treated with arsphenamine. The drug was given in doses of 10 mg. per kilo; each animal received six intravenous injections, one each at weekly intervals. Treatment was begun 181 to 194 days after inoculation, at a time when previous experience had shown that such animals would be refractory to a second inoculation. In all six of these animals the initial phenomena of syphilitic infection had by this time subsided and the animals were not showing any signs of activity of the disease. From 279 to 384 days after inoculation, the

animals in both treated and untreated groups were anesthetized with ether and the heart's blood aspirated with a sterile syringe and inoculated at once, without defibrinating and before clotting could take place, into the testes of two normal rabbits. Sometimes one testis, sometimes two were inoculated, depending upon the amount of blood aspirated, but never less than 1.0 cc. and frequently 1.5 cc. was injected into a single testis. The internal organs and the popliteal lymph nodes were then removed from the rabbit under aseptic precautions and portions of the former were emulsified in physiological salt solution and inoculated separately into one testis of each of two normal rabbits. The organs selected for transfer to normal rabbits were as follows: heart, liver, brain, spleen and bone marrow mixed, and the testicle which was originally inoculated and in which syphilitic orchitis had developed and subsided spontaneously. The popliteal nodes were emulsified in salt solution *in toto* and the entire amount of emulsion was inoculated into the testes of two normal rabbits. Thus, including the heart's blood, for each rabbit studied, seven pairs of normal rabbits were inoculated. In the case of the treated animals, tissue transfers were carried out 49, 65, 70, 76, 76, and 168 days after the last dose of arsphenamine.

There was one animal in the treated group in which syphilitic keratitis developed in one of the eyes both before and after treatment. At the time of organ transfer, 49 days after the last dose of arsphenamine, this keratitis was at its height. Accordingly, when the animal was etherized for organ transfer the cornea was excised, emulsified in physiological salt solution, and the emulsion was examined with dark-field illumination and also inoculated into the testes of two normal rabbits.

All the animals to which organs were transferred were kept under observation for a period of 90 days before discarding them as negative. We regard this period as ample for the particular strain of treponemata with which we are working, as it is a highly virulent one and has never in our experience failed to produce infection when inoculated intratesticularly in normal rabbits, the orchitis being manifest well within 90 days.

The results of the experiment are shown in Table I.

Table I shows that in the case of the untreated rabbits virulent treponemata were recovered from the lymph nodes of all four animals; from the heart's blood once, from the liver three times, the mixed spleen and bone marrow twice, the originally inoculated testicle three times, but in no instance from the brain or heart muscle. The recovery of the treponemata from the heart's blood of Rabbit 1, which was untreated, does not, of course, permit of any deductions as to the presence of the microorganisms in the tissues of that animal. The fact that the heart's blood was infectious 297 days after inoculation is of considerable interest from the standpoint of the possibility of

TABLE I.

Result of Organ Transfer from Untreated and Treated Syphilitic Rabbits to Normal Rabbits.

Organ.	Untreated group.				Treated group.					
	Rabbit No.				Rabbit No.					
	1	2	3	4	5	6	7	8	9	10
Heart's blood.	+ + (45) (52)	− −	− −	− −	− −	− −	− −	− −	− −	− −
Heart muscle.	− −	− −	− −*	− −	− −	− −	− −	− −	− −	− −*
Liver.	+ + (55) (48)	+ − (49)	− −	+ − (42)	− −*	− −	− −	− −*	− −	− −
Mixed spleen and bone marrow.	− + (45)	− + (65)	− −	− −	− −*	− −	− −	− −	− −	− −
Testicle.	+† (37)	+ + (25) (32)	− −	+ + (39) (39)	− −	− −	− −	− −	− −*	− −
Brain.	−* −*	− −	− −	− −	− −	− −	− −	− −	− −	−* −*
Lymph node.	+ + (29) (29)	−* + (36)	+ −* (50)	+ + (43) (47)	− −	− −	− −	− −	− −	− −
Cornea.										+ + (47) (54)

The figures in parenthesis indicate the day of appearance of syphilitic orchitis in the inoculated rabbits. Results are not reported as positive unless treponemata could be demonstrated. Each + or − sign refers to a single rabbit.

* The animal died before 90th day after inoculation.

† Only one animal inoculated.

fresh blood stream invasions late in the course of syphilitic infection. Neisser has recorded a similar result in apes. He demonstrated the infectiousness of the blood of one of his animals 283 days after inoculation (4).

The animals inoculated with lymph node or testicular emulsions developed syphilitic orchitis earlier and with greater uniformity than those inoculated with heart's blood or internal organs. Inasmuch as we have shown (9) that the richer the inoculum the shorter is the incubation period, it may be suspected that the inocula composed of lymph node or testicular emulsion contained more virus than did the inocula composed of heart's blood, liver, or mixed spleen and bone marrow.

In the case of the six treated animals, tissue transfer to normal animals was negative in every instance except one; namely, in the case of Rabbit 10. This animal, as stated previously, had keratitis prior to treatment. Following treatment keratitis reappeared in the same eye. The involved cornea, excised at the time that the other organs were removed, was emulsified in physiological salt solution and the resultant emulsion examined for *Treponema pallidum*. Several typical examples were seen with the aid of dark-field illumination and the emulsion was then inoculated into the testes of two normal animals with a subsequent positive result in each. In spite of the fact that the animal was not cured, as evidenced by a relapsing keratitis following treatment and the recovery of the organisms from the cornea, the internal organs appeared to have been sterilized by the treatment.

DISCUSSION.

It is generally agreed that as syphilitic infection develops in man, apes, or rabbits, the host gradually loses his susceptibility to a superimposed infection until finally there comes a time when it is impossible to bring about a successful second infection. This refractory state persists for a considerable time after the early manifestations of the disease have subsided. At first this refractory state was thought, from observations upon man, to represent a true acquired immunity similar to that seen after such acute infections as typhoid fever, and it was believed to indicate complete subsidence of the

infection. Neisser, however, was able to show that in apes this refractory state was associated with the presence of the first infection in the host. The ape infected with syphilis is able to overcome the local lesions in the course of time but is not able in most instances to rid its body of the infection, although it is refractory to a second infection. In this respect the behavior of the rabbit is quite similar to that of the ape.

Treatment of syphilitic rabbits with arsphenamine apparently does not influence this refractory state, acquired as a result of the infection. Both Kolle and ourselves have shown that when rabbits are treated with arsphenamine at a time when they are known to be refractory to a second infection, they remain refractory for an appreciable time after treatment has ceased. Is the maintenance of this refractory state dependent upon the persistence of the first infection in spite of treatment or has the original infection been eliminated by the aid of drugs? If it has been eliminated then the acquired refractory state may be conceived of as not necessarily dependent upon the persistence of the first infection but may survive its abolition.

It seems to us that our experiments favor the latter view. Of six syphilitic rabbits treated late in the course of the infection, at a time when they would be uniformly refractory to a second infection, the popliteal lymph nodes, heart's blood, and selected internal organs were found to be incapable of transmitting syphilitic infection to normal rabbits. In one of these six animals, in which keratitis occurred before and after treatment, the involved cornea remained infected in spite of treatment, although the lymph nodes and internal organs appeared to have been rid of the infection. In four control untreated animals the infection persisted in the lymph nodes and in many instances in one or more internal organs. Thus, while all four of the untreated animals continued to harbor the infection, five out of six of the treated animals, or 83.3 per cent, were entirely rid of their infection as judged by tissue transfer. It would appear then, under the conditions of the experiment, that when arsphenamine is administered to syphilitic rabbits late in the course of the infection, the lymph nodes and internal organs may be sterilized in the majority of instances. Judged by such a criterion, biological cure of syphilis is possible in the rabbit, even if the treatment be postponed 6 months or more after inoculation.

It must be admitted that the possibility exists that there remain in other untested tissues of these treated animals foci of syphilitic infection which have not been reached by the arsphenamine. Again, it may be that in the tested organs of the treated animals the infection has been suppressed to the point where it is no longer capable of being transmitted to normal animals but has not been completely eradicated. Such might be the case if there were resistant forms in the life cycle of the parasite. In answer to the first of these objections, we can say that we have selected for study representative tissues, which in the light of previous experience of other workers and of our knowledge of the course of syphilitic infection in man, might reasonably be expected to harbor the infection. There are obvious limits to the tissue transfer method. It is manifestly impossible to emulsify an entire rabbit and inoculate it into normal rabbits, hence, it is extremely difficult, if not impossible, absolutely to exclude syphilitic infection in an animal. In answer to the second objection it may be said that it would be difficult to recognize the existence of an active syphilitic infection in a tissue that gave no macroscopic evidence of disease and yielded only negative results on inoculation. Not even the demonstration of spirochetes in such tissue by histological methods would guarantee the viability of those microorganisms. As yet no detailed histological studies of the organs of the animals in this experiment have been made. They were all free of gross evidence of syphilitic infection.

If it be concluded, from the results of tissue transfer as carried out in this experiment, that treatment of rabbits with arsphenamine late in the course of the infection brings about a biological cure in most instances, then it must be concluded that failure to reinoculate a treated syphilitic rabbit cannot be interpreted as evidence of the persistence of the first infection. In other words, the reinoculation test in such an animal could not be utilized as a criterion of cure, but rather as an index of resistance only. Furthermore, as our experiments perhaps indicate, such a resistance may, in the case of the rabbit, persist after the elimination of the first infection. Whether such a state of resistance to second infection in the absence of the first infection is to be regarded as true immunity or not, will depend entirely upon one's definition of that term. It matters little whether

one speaks of such a state as one of immunity or refractoriness. What is important is to realize that it is a property which the animal body acquires as a result of syphilitic infection, and failure to acquire it can be governed by proper selection of the time at which arsphenamine is given. It is the expression of a biological reaction between host and parasite and if that reaction is interfered with before there has been time for its consummation, the refractory state does not appear. Indeed, there is evidence to show that the animal may be rendered less resistant (10). On the other hand, if the animal is allowed to react against the invading organism without interference in the shape of therapeutic agents, so that the primary orchitis runs its course and the pathological process subsides by virtue of the inherent defensive mechanism of the host, then this biological reaction is completed and the refractory state is established. Treatment at this point will not alter it.

Whether or not the establishment of the refractory state results in eradication of the infection is another matter, likewise to what extent the persistence of the infection is necessary for the indefinite maintenance of the refractory state is still perhaps debatable. The point which we wish to emphasize is, that, in our opinion, it is the opportunity which is afforded the host of reacting against the syphilitic infection without extraneous aids which determines his acquisition of the refractory state and his behavior toward a second infection, rather than the persistence of the infection.

The experiments recorded in this and the previous papers in the series offer evidence which strongly suggests that the refractory state is capable of surviving the abolition of the infection. If this is true, the reinoculation test must be regarded as no valid criterion of cure, and, accordingly, the prospect of curing syphilis in the rabbit becomes more hopeful than the deductions drawn from recent experimental work based upon the success or failure of reinoculation, would indicate.

If the reinoculation test cannot be accepted as a criterion of cure, the question may be raised, to what extent can the sterility of lymph nodes following treatment be regarded as an index of cure? We have too few experiments to permit of generalizations on this point. In the untreated animals the popliteal lymph nodes were infectious in every instance in which the internal organs were infectious. On

the other hand, these nodes were infectious in one instance when the above mentioned tissues were non-infectious. Among the treated animals, however, there was one in which keratitis recurred *after* treatment, and in this animal the lymph nodes were non-infectious, while the involved cornea was proved to be infectious. It would appear from this observation that sterility of lymph nodes does not in every instance indicate that the animal is free of syphilitic infection, particularly if there has been a preexisting keratitis. Lymph node sterility is, therefore, no absolute indication of cure of syphilis and perhaps the same may be said of the internal organs, but it is probably the best single index that we have. We incline to the view that study of the lymph nodes or internal organs of treated animals is essential in the ultimate evaluation of any chemotherapeutic agent in experimental syphilis, but continued observation of the treated animals to detect recurrent syphilitic lesions is fully as important and cannot be neglected. Mere observation of the behavior of the initial lesion as well as the disappearance of the treponemata from it is obviously insufficient for the purpose.

SUMMARY.

Simultaneous transfers to the testes of normal rabbits of circulating blood, heart muscle, liver, brain, spleen and bone marrow (mixed), inoculated testicle, and popliteal lymph nodes from a series of untreated syphilitic rabbits, demonstrated the persistence of the original infection uniformly in the lymph nodes and less regularly in the liver, mixed spleen and bone marrow, and testis originally inoculated. In one instance the circulating blood was found to be infectious. Transfer of similar tissues from syphilitic rabbits treated with arsphenamine late in the course of the disease failed to disclose syphilitic infection of any of these tissues. In one animal, in which keratitis developed both before and after treatment, the blood, internal organs, and lymph nodes were found to be non-infectious in spite of the fact that the cornea was shown to be the site of a syphilitic inflammation. Transfer of lymph nodes or internal organs of treated syphilitic rabbits is probably the best method of evaluating an antisyphilitic agent, but it must be supplemented by careful observation of treated animals over an appreciable interval of time following treatment.

The results of this study support the idea that failure to reinoculate a treated syphilitic animal does not necessarily mean the existence of the first infection but might be interpreted as indicating the presence of an acquired resistance which persists in rabbits in which no trace of the first infection can be demonstrated.

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BLOOD SUGAR STUDIES.

I. Rapid Alterations in the Blood Sugar Level of Rabbits as Result of Intravenous Injections of Killed Bacteria of Various Types.

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The effect produced by bacteria on the metabolism of the body into which they are introduced is an almost unexplored field of investigation. While many changes associated with infection, such as increased heat production, changes in functional activity of the circulatory and excretory systems, leucocytosis, and the development of immune bodies, have been elaborately investigated, little attention has been paid to the direct chemical changes resulting from bacterial invasion. The metabolic products of the bacteria, the enzymes they elaborate, the chemical composition of the bacterial body itself, aside from the specific toxins of the particular organism, must exert profound effects upon the chemistry of the body of the host. Recently Menten and Manning (1), in producing hyperglycemia in animals by the injection of killed organisms of the enteritidis-paratyphoid B group, have shown the possibilities of experimental work in this direction.

In order to investigate one phase of this problem, experiments were conducted to determine what effect on the blood sugar level of rabbits was produced by injecting intravenously killed organisms of various types. Any effect that would be produced would then be due to the chemical composition of the bacterial body, since killed organisms were used, thus eliminating the factor of the biological activity of the bacteria. It was found that certain organisms caused an immediate rise in blood sugar to a high level, while other organisms produced no effect.

Experimental Procedure.

18 hour cultures of various organisms on agar were suspended in physiological salt solution, killed by heat, and 1 cc. of the suspension, containing usually approximately 2 billion organisms, was injected intravenously into young adult male rabbits. Blood was withdrawn from the ear veins immediately before injection and at short intervals thereafter. Blood sugar determinations were made by the Folin-Wu method and leucocyte counts were made on each sample of blood. In cases where the blood pressure became extremely low, blood was obtained by heart puncture. In many cases the rectal temperature was taken immediately before each sample of blood was withdrawn.

In the following experiments a marked hyperglycemia was produced.

Experiment 1. *B. proteus.*—*B. proteus*, recently isolated from the urine of a patient with cystitis, was suspended in salt solution, killed, and injected intravenously. See curve of Fig. 1.

Time.		Blood sugar.
		per cent
Nov. 20, 1924		
a.m.		
10.45	Before injection.	0.104
11.00	*B. proteus* injected.	
11.15		0.137
11.30	Vasoconstriction and great weakness.	0.163
11.45		0.188
p.m.		
12.05	Animal prostrated.	0.253
12.20		0.275
12.50		0.264
1.50	" recovering.	0.269
5.00		0.146
6.00	" apparently well.	0.145

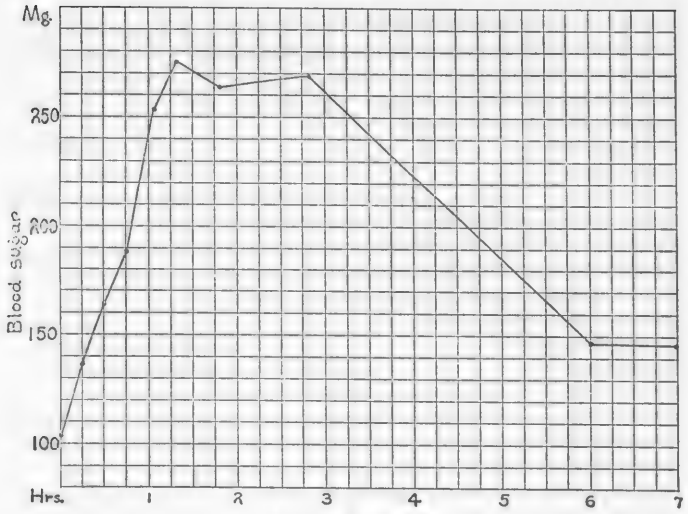

FIG. 1. *B. proteus.*

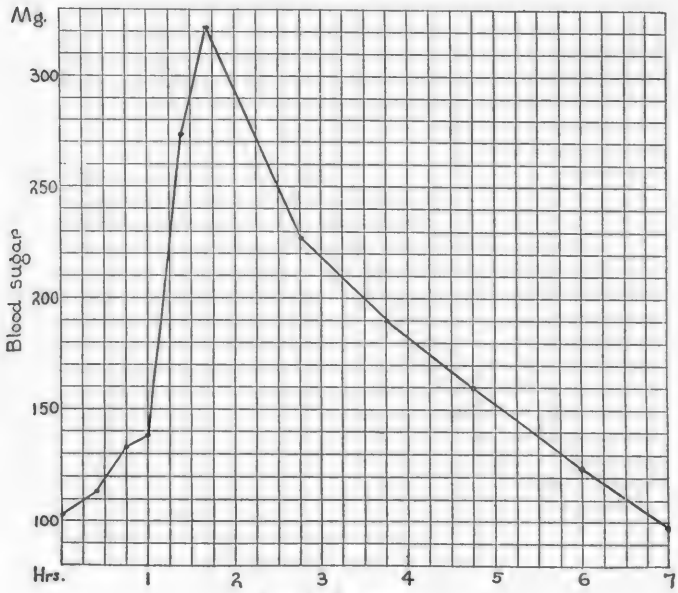

FIG. 2. *B. proteus.*

Experiment 2. *B. proteus.*—A large dose of killed *B. proteus* was given to the same rabbit used in Experiment 1, 1 week later. For curve, see Fig. 2.

Time.		Blood sugar.	W.B.C.
		per cent	
Nov. 28, 1924			
a.m.			
10.45	Before injection.	0.101	5,500
11.00	*B. proteus* injected.		
11.25		0.114	4,800
11.42	Vasoconstriction of ears.	0.134	2,200
m.			
12.00	Animal becoming weak.	0.138	1,900
p.m.			
12.25		0.274	1,200
12.40	Prostration.	0.321	1,250
1.45		0.227	600
2.45		0.190	700
3.45	Animal better.	0.155	1,600
5.00		0.124	3,800
6.00	" well.	0.097	4,700

Experiment 3. *B. proteus vulgaris.*—A stock culture of *B. proteus vulgaris*, which had been growing on artificial media for a long time, was used.

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Feb. 28, 1925				
a.m.				
8.45	Before injection.	0.095	8,600	101.2
9.00	*B. proteus vulgaris* injected.			
9.15		0.097	1,800	101.4
9.35	Vasoconstriction, copious diarrhea.	0.113	1,800	103.4
9.55		0.106	1,200	103.2
10.15		0.127	2,100	102.4
10.30		0.118	2,350	101.7
11.00	Animal prostrated.	0.147	1,600	101.0
m.				
12.00		0.118	1,650	100.4
p.m.				
1.00	" better.	1.108	2,900	101.3
2.00	" lively.	0.108	3,200	102.2

Experiment 4. B. coli.—B. coli communis, recently isolated from normal human feces, was suspended in salt solution, killed, and injected intravenously. See Fig. 3.

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 14, 1925			
a.m.			
10.00	Before injection.	0.117	5,600
10.20	*B. coli communis* injected.		
10.35		0.117	1,600
10.50		0.129	800
11.05		0.132	600
11.20		0.158	1,400
11.35		0.175	1,800
11.50	Animal prostrated.	0.244	1,400
p.m.			
12.30		0.182	1,400
1.30		0.132	1,600
2.30		0.120	1,200
3.30		0.116	2,400
4.30	" well.	0.116	4,600

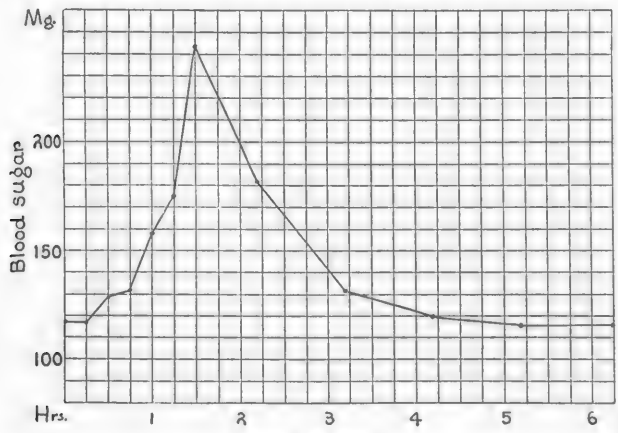

FIG. 3. *B. coli.*

Experiment 5. B. coli.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Mar. 27, 1925				
a.m.				
10.00	Before injection.	0.097	6,400	103.2
10.30	Injection of same organism as in Experiment 4, grown in media 2 mos.			
10.50		0.099	1,800	104.0
11.05		0.098	2,250	104.0
11.25		0.105	1,300	104.6
11.40		0.141	1,120	104.8
11.55		0.160	1,000	104.6
p.m.				
12.25		0.176	450	104.6
1.00		0.161	650	103.0
2.00		0.145	700	102.2
3.00		0.169	650	101.6
4.00		0.165	850	
6.00		0.112	3,650	

Experiment 6. B. paratyphosus B.—A stock culture of *B. paratyphosus* B was used. See Fig. 4.

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 27, 1925			
a.m.			
9.00	Before injection.	0.104	5,600
9.10	*B. paratyphosus* B injected.		
9.25		0.114	2,200
9.40		0.114	2,200
9.55	Animal weak.	0.104	1,700
10.10		0.130	1,300
10.25		0.160	1,600
10.45	" prostrated.	0.208	1,800
11.15		0.200	1,800
11.45		0.170	1,800
p.m.			
12.45		0.157	
1.45		0.113	3,000
2.45	" well.	0.090	4,800

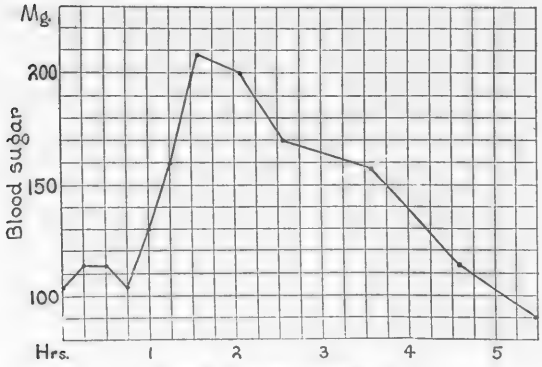

FIG. 4. *B. paratyphosus* B.

Experiment 7. *B. paratyphosus B.*—See Fig. 5.

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Mar. 10, 1925				
a.m.				
8.00	Before injection.	0.088	5,500	102.4
8.30	*B. paratyphosus* B injected.			
8.45		0.097	1,400	101.8
9.10	Diarrhea, weak.	0.114	700	101.6
10.00		0.187	850	101.4
10.30	Prostrated.	0.196	800	100.8
11.45		0.150	1,000	98.0
p.m.				
1.00		0.125	1,800	99.0
2.30		0.108	2,600	100.2
4.20	Recovery.	0.090	5,000	

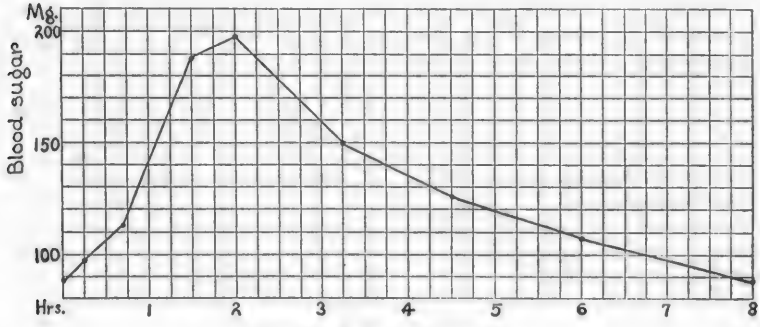

FIG. 5. *B. paratyphosus* B.

In the following experiments, a slight irregular rise in blood sugar occurred.

Experiment 8. *B. paratyphosus A.*—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Feb. 17, 1925				
a.m.				
10.00	Before injection.	0.097	5,600	103.2
10.15	*B. paratyphosus* A injected.			
10.30		0.102	1,200	103.0
10.45		0.113	1,600	103.4
11.00	No symptoms.	0.098	1,200	103.8
11.15		0.098	1,000	103.6
11.30		0.120	1,200	103.6
m.				
12.00		0.110	1,600	103.4
p.m.				
2.30		0.099	3,600	103.4

Experiment 9. *B. paratyphosus A.*—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Apr. 15, 1925				
m.				
12.00	Before injection.	0.096	4,400	103.2
p.m.				
1.15	*B. paratyphosus* A injected.			
1.30		0.096	2,400	103.0
1.45		0.101	1,600	104.0
2.00	Fall in blood pressure.	0.105	1,400	104.2
2.15	Slight weakness.	0.125	1,300	104.6
2.45		0.133	800	103.3
3.15		0.141	700	103.6
3.45		0.124	1,100	103.6
4.45		0.125	1,600	103.4

Experiment 10. B. enteritidis of Gaertner (Lethal Dose).—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Feb. 17, 1925				
a.m.				
8.30	Before injection.	0.108	6,600	104.6
8.45	*B. enteritidis* of Gaertner injected.			
9.00		0.097	2,600	104.8
9.15	Weak; low blood pressure.	0.123	2,000	105.2
9.40	Copious diarrhea, prostration.	0.132	1,600	105.4
10.00		0.120	900	105.2
10.15			2,600	104.6
10.20	Shallow, rapid respirations.	0.132	2,800	104.0
10.22	Dead.	0.122		

Experiment 11. B. enteritidis of Gaertner.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Apr. 15, 1925				
a.m.				
10.10	Before injection.	0.093	15,200	102.8
10.45	*B. enteritidis* of Gaertner injected.			
11.00		0.089	1,100	102.4
11.15		0.084	1,400	102.6
11.37	Marked prostration.	0.083	950	
11.50		0.082	1,300	103.2
p.m.				
12.15		0.088	700	102.4
12.35		0.091	2,200	101.4
1.00	Recovery.	0.095	2,000	100.8

The following organisms produced no notable fluctuations in the blood sugar level.

Experiment 12. B. fæcalis alkaligenes.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Feb. 25, 1925				
a.m.				
10.30	Before injection.	0.185	9,000	101.4
11.00	*B. fæcalis alkaligenes* injected.			
11.15		0.161	2,200	101.6
11.30		0.178	2,400	101.8
11.50	Weak.	0.196	1,800	101.1
p.m.				
12.15		0.188	1,600	100.0
12.45		0.187	1,700	99.3
1.30	Recovery.	0.185	3,600	100.1

Experiment 13. Streptococcus hæmolyticus.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Feb. 25, 1925				
a.m.				
9.30	Before injection.	0.097	4,100	103.2
9.45	*Streptococcus hæmolyticus* injected.			
10.15		0.101	4,300	103.3
10.45	No symptoms.	0.106	7,600	103.4
11.15		0.106	2,200	103.6
p.m.				
12.45		0.101	4,600	103.6
2.30		0.103	7,000	103.0

Experiment 14. Streptococcus viridans.—

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 19, 1925			
a.m.			
10.30	Before injection.	0.075	7,600
10.45	*Streptococcus viridans* injected.		
11.00		0.066	3,200
11.15			2,800
11.30		0.072	3,600
11.45	No symptoms.	0.072	2,400
m.			
12.00		0.073	3,000
p.m.			
12.15		0.072	2,600
12.30		0.072	2,600
1.00		0.072	3,800
2.15		0.072	6,600
3.15		0.066	5,800

Experiment 15. Streptococcus viridans.—

Time.		Blood sugar.	W.B.C.
		per cent	
Mar. 16, 1925			
a.m.			
10.00	Before injection.	0.091	4,400
10.05	*Streptococcus viridans* injected.		
10.10		0.114	3,000
10.20	No symptoms.	0.108	1,800
10.30		0.098	1,400
10.40		0.090	1,600

Experiment 16. Staphylococcus aureus.—

Time.		Blood sugar.
		per cent
Dec. 1, 1924		
a.m.		
9.00	Before injection.	0.109
9.15	*Staphylococcus aureus* injected.	
9.30		0.115
9.45		0.125
10.00	No symptoms.	0.109
10.15		0.108
10.30		0.126
10.45		0.113

Experiment 17. Staphylococcus aureus.—

Time.		Blood sugar.
		per cent
Feb. 3, 1925		
a.m.		
8.30	Before injection.	0.124
8.45	*Staphylococcus aureus* injected.	
9.00		0.125
9.15	No symptoms.	0.124
9.45		0.125

Experiment 18. B. pyocyaneus.—

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°F.
Mar. 23, 1925				
a.m.				
11.30	Before injection.	0.096	7,500	101.6
11.45	*B. pyocyaneus* injected.			
m.				
12.00		0.087	2,600	103.2
p.m.				
12.20		0.090	1,600	104.6
12.40	Weak.	0.083	1,500	
1.05		0.099	600	105.8
1.30		0.090	1,800	104.2
2.05	Recovery.		1,800	105.2

Experiment 19. B. typhosus.—

Time.		Blood sugar.	W.B.C.	Temperature.
a.m.		*per cent*		°F.
9.15	Before injection.	0.099	6,600	102.0
9.30	*B. typhosus* injected.			
9.45		0.099	3,900	102.0
10.00		0.099	2,400	102.8
10.15	No symptoms.	0.086	2,100	103.4
10.40		0.099	2,200	103.8
11.00			2,000	103.8
11.30			2,140	103.8

In instances giving no rise in blood sugar, systemic reactions were in some cases severe, while in other cases, as after staphylococci and streptococci, practically no symptoms were manifested. These negative results served as controls for the positive results, as the animals were exposed to the same stress of handling and bleeding. The slight fluctuations in blood sugar during the course of these negative experiments were negligible in comparison to the regular curves of marked increase in the positive cases.

Organisms producing hyperglycemia are less effective after prolonged growth on artificial media. The saline suspension must be used promptly, as, after standing, a change of some sort occurs which renders it ineffective in causing hyperglycemia. This was accidentally noted in using an old saline suspension of *Bacillus coli* and of *Bacillus paratyphosus* B which had no effect on the blood sugar, but which had previously, when freshly made, caused marked hyperglycemia.

The leucocyte counts were made merely to give an index of the depressed state of the animal and to show that the fall in leucocytes occurred simultaneously with the rise in blood sugar. The recovery of leucocytes lagged behind the return to normal of the blood sugar level. The fluctuations in temperature appeared to have no relation to the blood sugar curve or leucocyte count.

DISCUSSION.

The rapid rise in the blood sugar level produced by injections of killed organisms of the type of *Bacillus proteus*, *Bacillus coli*, and *Bacillus paratyphosus* B was followed by a return to normal in a few hours. A slight irregular rise occurred with *Bacillus enteritidis* of Gaertner and *Bacillus paratyphosus* A.

The results with *Bacillus enteritidis* and *Bacillus paratyphosus* B are in accord with the results of Menten and Manning (1) (1924), who found a rise in the blood sugar of rabbits spontaneously infected with organisms of the enteritidis-paratyphoid B group. After injecting intravenously killed organisms of these types, they found a rise in blood sugar which attained a maximum of 130 to 160 mg. and returned to normal in a few hours. Lethal doses produced a

rise to 235 to 250 mg. followed by a decrease until a low value of 40 to 50 mg. was reached at time of death.

In our experiments the hyperglycemia produced by various organisms was in general of higher degree than in the experiments of Menten and Manning. At no time have we obtained a hypoglycemia, as they did.

Evidence that the mechanism of the production of these changes in the blood sugar level is by increased glycogenolysis, probably due to sympathetic stimulation, will be presented in a subsequent paper.

SUMMARY.

A rapid rise in the blood sugar level of rabbits was produced by intravenous injections of killed *Bacillus proteus*, *Bacillus coli*, and *Bacillus paratyphosus* B, which returned to nearly the previous level in a few hours time.

A less pronounced rise in blood sugar was produced by killed *Bacillus paratyphosus* A and *Bacillus enteritidis*.

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BLOOD SUGAR STUDIES.

II. Blood Sugar Changes in Fatal Bacterial Anaphylaxis in the Rabbit.

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Chemical studies of anaphylaxis have been directed more toward the agents causing anaphylaxis than to the chemical reactions of the body resulting from this condition. Certain chemical changes associated with this condition have been investigated, involving, for example, the blood chlorides (Zunz and La Barre (1)), antitrypsin content of the blood (Ando (2)), substances excreted in the urine (Pfeiffer (3)), serum proteins and ferments of the blood (Jobling (4)), protein destruction (Major (5)), amino acids (Auer and Van Slyke (6)), and blood urea nitrogen (Hisanobu (7)). Interest has been directed chiefly toward protein rather than carbohydrate metabolism.

With a view of determining any changes in the blood sugar level, bacterial anaphylaxis was produced in rabbits, by giving several sensitizing injections of killed organisms and then allowing a period of 6 to 8 weeks to elapse, when the injection was given which induced anaphylaxis. Organisms were used which in themselves produced changes in the blood sugar level of unsensitized animals and also those which had no such effect (8).

A. The effect on the blood sugar level of the production of anaphylaxis by organisms which have no effect on the blood sugar level of the unsensitized animal is given in the following experiments.

Experiment 1. Anaphylaxis (B. fæcalis alkaligenes).—See Fig. 1.

Time.		Blood sugar.	Temperature.
		per cent	°*F.*
Mar. 29, 1925			
p.m.			
2.45	Before injection.	0.075	102.2
3.10	*B. fæcalis alkaligenes* injected.		
3.40		0.076	100.3
4.10		0.106	99.8
4.27		0.195	99.4
4.35		0.227	
4.55	Death.	0.400	

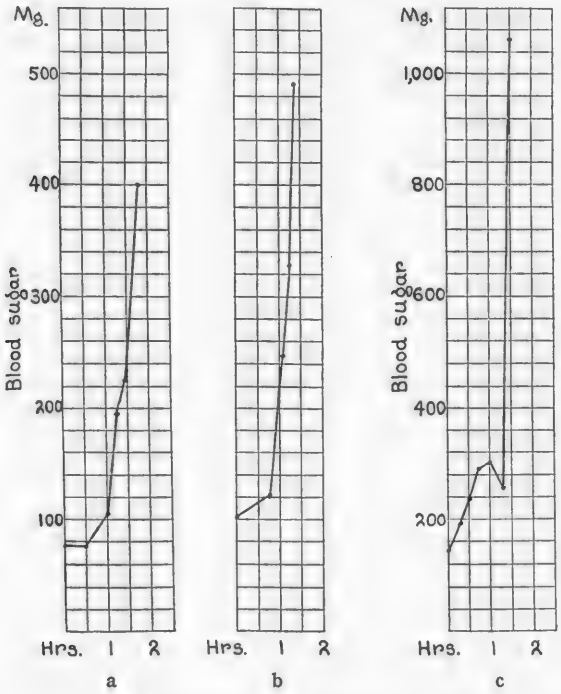

FIG. 1, *a* to *c*. (*a*) Experiment 1. Anaphylaxis (*B. fæcalis alkaligenes*). (*b*) Experiment 2. Anaphylaxis (*Streptococcus viridans*). (*c*) Experiment 3. Anaphylaxis (*Streptococcus viridans*).

Experiment 2. Anaphylaxis (Streptococcus viridans.)—See Fig. 1.

Time.		Blood sugar.
		per cent
Mar. 24, 1925		
a.m.		
10.55	Before injection.	0.103
11.00	*Streptococcus viridans* injected.	
11.45		0.122
p.m.		
12.10	Restless.	0.247
12.13	Convulsions, gasping respiration.	0.326
12.15	Death.	0.490

Experiment 3. Anaphylaxis (Streptococcus viridans).—See Fig. 1.

Time.		Blood sugar.	W.B.C.
		per cent	
Jan. 16, 1925			
a.m.			
10.30		0.146	4,000
10.45	*Streptococcus viridans* injected.		
11.00		0.191	1,600
11.15		0.239	1,400
11.30		0.286	1,800
11.45		0.306	1,000
m.			
12.00		0.253	800
p.m.			
12.18	Death.	1.060	2,000

B. The effect on the blood sugar level of the production of anaphylaxis by organisms which have a slight effect on the blood sugar level in the unsensitized animal is given in the following experiments.

Experiment 4. *Anaphylaxis* (*B. enteritidis of Gaertner*).—See Fig. 2.

Time.		Blood sugar.	W.B.C.
		per cent	
Feb. 13, 1925			
a.m.			
9.20	Before injection.	0.132	7,500
9.30	*B. enteritidis* of Gaertner injected.		
9.45		0.130	2,700
10.00		0.122	2,200
10.15		0.131	1,700
10.35		0.156	1,200
10.42		0.190	6,000
10.44	Death.	0.345	5,600

Experiment 5. *Anaphylaxis* (*B. paratyphosus A*).—See Fig. 2.

Time.		Blood sugar.
		per cent
Mar. 29, 1925		
p.m.		
1.25	Before injection.	0.099
1.32	*B. paratyphosus* A injected.	
2.05		0.084
2.20		0.111
2.23		0.186
2.24		0.188
2.25	Death.	0.230

C. The effect on the blood sugar of the production of anaphylaxis induced by organisms which produce a marked hyperglycemia in the unsensitized animal, is given in the following experiments.

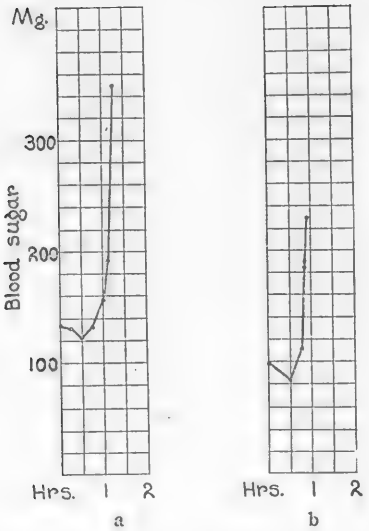

FIG. 2, *a* and *b*. (*a*) Experiment 4. Anaphylaxis (*B. enteritidis*). (*b*) Experiment 5. Anaphylaxis (*B. paratyphosus* A).

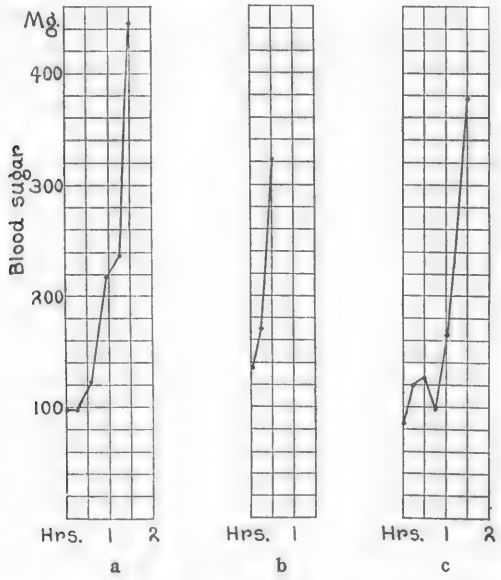

FIG. 3, *a* to *c*. Anaphylaxis (*B. coli*). (*a*) Experiment 6. (*b*) Experiment 7. (*c*) Experiment 8.

Experiment 6. *Anaphylaxis* (*B. coli*).—See Fig. 3.

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Mar. 23, 1925				
a.m.				
9.05	Before injection.	0.098	5,500	102.4
9.15	*B. coli* injected.			
9.30		0.098	2,600	102.2
9.50		0.123	1,800	102.6
10.10		0.218	2,800	102.2
10.25		0.238	2,300	102.2
10.45		0.444	5,800	
10.48	Death.			

Experiment 7. *Anaphylaxis* (*B. coli*).—See Fig. 3.

Time.		Blood sugar.	W.B.C.
		per cent	
Dec. 5, 1924			
p.m.			
3.45	Before injection.	0.134	12,800
4.15	*B. coli injected.*		
4.30		0.168	1,300
4.45	Death.	0.321	3,200

Experiment 8. *Anaphylaxis* (*B. coli*).—See Fig. 3.

Time.		Blood sugar.	W.B.C.	Temperature.
		per cent		°*F.*
Mar. 10, 1925				
a.m.				
11.00	Before injection.	0.084	8,000	102.8
11.05	*B. coli* injected.			
11.20		0.120	1,600	102.6
11.35		0.127	800	103.6
11.50		0.099	600	104.2
p.m.				
12.05		0.167	800	104.3
12.30	Death.	0.377	2,500	

It will be seen from these results that in general there is a gradual rise in blood sugar during the latent period before symptoms of anaphylaxis are manifested. Then, when the animal becomes restless, prostrated, with fall of blood pressure, and develops convulsions and becomes dyspneic, there is a more rapid rise in the blood sugar which reaches an unusually high level at time of death. Often the final sharp ascent is preceded by a short downward curve of relatively lower blood sugar.

There is no material difference in the blood sugar curve in anaphylaxis produced by organisms which in themselves have an effect on the blood sugar and by those which have no such effect on the unsensitized animal. The height of the blood sugar seems to depend on the degree of hypersensitivity of the animal rather than on the agent producing it.

GENERAL DISCUSSION.

In considering the mechanism by which rapid alterations in the sugar content of the blood are produced by the introduction of killed bacteria intravenously in sensitized and unsensitized rabbits, two possibilities are to be considered: (1) whether there is a failure in the breaking down of glucose and its utilization, with consequent accumulation in the blood of glucose which is being thrown into the blood stream in normal amounts; or (2) whether there is a sudden mobilization of glycogen, and the increase in the blood sugar level represents increased glycogenolysis, and hence an increase in the amount of sugar thrown into the blood stream.

Failure in the breaking down and utilization of glucose can be conceived of as being produced in two ways:

(*a*) As result of a general depressed state of all metabolic activities including depression of those cells functioning to break down and utilize glucose. If this were the case there would be an agonal rise in blood sugar in every case in which the moribund state is prolonged. This, however, does not occur, and hence this possibility can be excluded.

(*b*) Through injury to special cells controlling the utilization of sugar. Menten and Manning (9) stress the importance of histological changes in the islands of Langerhans, together with changes in the

liver, spleen, and kidneys, in explaining the increase in blood sugar in their experiments after the injection of organisms of the paratyphoid B-enteritidis group. It does not seem likely to us that injury to the islets with resultant suspension in the activity of these cells can fully account for such marked hyperglycemia as developed in our experiments after a single injection of organisms. Usually transient functional changes are not registered as anatomical changes that can be detected by ordinary histological methods. It requires a more permanent injury to produce anatomical changes. To be responsible for such high blood sugar levels, the cells would have to be profoundly injured, and it is inconceivable that the cells could be restored to normal function in a few hours time if the injury was profound.

In the absence of indications that the rise in blood sugar is due to interference with utilization of sugar, the alternative mechanism, a sudden increased production of sugar, remains to be considered.

Hyperglycemia secondary to increased glycogenolysis can be produced by various well known methods.

The hyperglycemia caused by adrenalin is apparently produced by this mechanism. Macleod (10) showed that stimulation of the sympathetic nerves to the liver caused rapid mobilization of glycogen and hyperglycemia. The intact adrenals were found to be necessary for this effect of sympathetic stimulation. Griffith (11) has shown that hyperglycemia of nervous origin may be produced reflexly by stimulation of the sciatic and vagus nerves. Henderson and Underhill (12) have emphasized the effect of acapnia in mobilizing glycogen of the liver with resultant hyperglycemia. That asphyxia is potent in producing hyperglycemia has been evidenced by many investigators. Cannon (13) has stressed the emotions of rage and fear in producing stimulation of the sympathetic nervous system and hyperglycemia.

In our experiments, the picture had every appearance of sympathetic effect. The rapidity of development of the hyperglycemia, the associated vasomotor phenomena in the animals, and the swift restoration of equilibrium indicated a temporary effect, as one produced by nervous stimulation. Actual increase in the sugar discharged into the blood stream could alone account for the high levels attained.

Proof of this hypothesis was furnished by the fact that microchemical determinations showed disappearance of glycogen in the

livers of animals dying in anaphylaxis, in contrast to the abundant glycogen present in the normal animal. Furthermore, O'Neill, Manwaring, and Moy (14) have recently shown that in serum anaphylaxis produced in dogs, the liver is almost completely depleted of glycogen.

If sympathetic stimulation of glycogenolysis is the mode of production of the hyperglycemia in the present experiments, the question remains how this sympathetic stimulation is effected. Is it by the chemical stimulation of some constituents of the bacterial body—or, in the defence reaction of the body in response to the introduction of foreign bacteria, are substances elaborated by the tissues which exert a stimulating effect upon the sympathetic nervous system? The fact that the injection of any organism causes hyperglycemia in the animal sensitized to it suggests that the animal furnishes the stimulating substance rather than the bacterium. If so, why do certain organisms elicit this substance in unsensitized animals, while other organisms do not? The solutions to these questions remain to be found.

In anaphylaxis the effect of asphyxia, which is well known to cause hyperglycemia, may play a rôle in the production of glycogenolysis. The extreme heights in blood sugar are reached when there is violent respiratory difficulty, although the sugar rises markedly before any symptoms of asphyxia are manifested. This effect of asphyxia probably augments the effect of sympathetic stimulation. In the hyperglycemia produced by organisms in the unsensitized animal, asphyxia plays no rôle, as there is no respiratory difficulty in these animals.

The fact that the curve of blood sugar in anaphylaxis was the same whether organisms were used which caused hyperglycemia in the unsensitized animal or whether organisms were used which had no effect in the unsensitized animal, suggests that the mechanism of the production of these blood sugar changes is the same in anaphylaxis as in the reaction to bacteria in the unsensitized animal. If a certain type of mechanism controlled the one and another type the other, two effects would be superimposed in the case in which anaphylaxis was produced by organisms such as *Bacillus coli*, and there would be an augmentation in the height of the curve as compared with that of

anaphylaxis produced by such organisms as streptococci. No such augmentation occurred in our experiments. In fact, the opposite occurred, that is, the highest rise, of a phenomenally high value, occurred in anaphylaxis produced by *Streptococcus viridans*, which had no effect whatever on the blood sugar of the unsensitized animal.

In anaphylaxis, apparently, the degree of hypersensitivity of the animal determines the height of the blood sugar, not the agent used in producing it.

In searching the literature for previous work along these lines, a recent preliminary report was noted of experiments by McCullough (15), in which, during serum anaphylaxis in the dog, a slight increase in blood sugar was found, up to 0.27 per cent. It is of interest that the values were not as high as in our experiments, because in the dog, in which changes in the liver apparently play a conspicuous rôle in anaphylaxis (Manwaring (16)), one might expect a relatively great disturbance in the glycogen output. Petersen, Jaffe, Levinson, and Hughes (17), in experiments undertaken for the purpose of demonstrating endothelial permeability in anaphylaxis in the dog, determined various constituents of the thoracic duct lymph. Though they were interested chiefly in other chemical substances, sugar determinations were made in some experiments, and their tables show a distinct increase in the lymph sugar in anaphylaxis.

SUMMARY.

1. During bacterial anaphylaxis there is a gradual rise in the blood sugar level, which attains an extremely high value at the time of death.

2. The curve of blood sugar is quite similar whether anaphylaxis is induced by organisms which affect the blood sugar in the unsensitized animal or by organisms which have no such effect.

3. No instances occurred in which there was not a marked hyperglycemia in anaphylaxis.

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ORGAN WEIGHTS OF NORMAL RABBITS.

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The work carried out in these laboratories over a period of more than 10 years has emphasized the necessity for devoting more attention to the factor of the animal organism in the study of medical problems. In the fall of 1921, we began a systematic study of the animal organism in relation to disease which included parallel observations on normal rabbits and on rabbits inoculated with *Treponema pallidum* or with a malignant tumor. The study of normal rabbits was intended, in part, to serve as a control for the other experiments and, in part, to provide material for a comprehensive study of the mass relations of the various organs with especial reference to the subjects of variation, the effects of environmental influences, and the relation of these conditions to phenomena of animal resistance or the occurrence and general course of disease. These investigations are still in progress but they have reached a stage where it is possible to begin a correlation of results in a satisfactory manner.

This study of the normal animal was not planned primarily with a view to determining normal standards under what might be regarded as the most acceptable conditions but for the purpose of obtaining values applicable to the particular conditions that it was necessary for us to employ in the conduct of other experiments. Nevertheless it is believed that the results obtained are sufficiently accurate for all practical purposes and that they provide reliable information concerning conditions that obtain in normal stock rabbits.

Preliminary reports have been published covering several aspects of this work but no detailed reports have been made (1). These will appear as opportunity permits. The present paper will be limited to a report on the weights of organs without reference to the relation

of one organ to another or to any factor that may affect the weight of individual organs or their relations to one another. These subjects will be reserved for future consideration.

Methods and Material.

The results reported in this paper are based on a study of 350 *male* rabbits killed and examined between January 1, 1922, and July 1, 1924.

The rabbits comprising the series represent carefully selected stocks of well nourished animals, free from any external evidence of disease. They were obtained from numerous breeders or dealers who draw their stock chiefly from New York, New Jersey, Pennsylvania, Delaware, and Maryland. A few animals came from some of the New England states but the great majority of them came from eastern Pennsylvania and the immediate vicinity of New York City. The series includes all of the breeds of rabbits that are commonly used and in essentially the same proportions as such breeds have been used in our laboratory for other experiments. The exact age of the animals was unknown in most instances but the great majority of them were between 6 months and 2 years old.

The plan followed in carrying out the investigation was essentially the same as that used in the conduct of other experiments. Animals were selected from the general stock and placed in individual cages for observation. The period of observation varied (in individual instances) from a few days to as long as a year but in most instances was from 2 weeks to 2 months, the exact time being determined by experiments for which these animals served as controls. While under observation, the animals were kept in large, well lighted, and well ventilated rooms with a southern exposure. The rooms were heated and the temperature was maintained at a relatively constant level of 60–70°F. during the colder months of the year (October to May). The cages and bedding were kept scrupulously clean. The animals were fed in accordance with a regular system of feeding employed in these laboratories. Their diet at all seasons consisted of hay, oats, and cabbage or carrots of the best quality obtainable.

The records kept included the source of the animal, the date of receipt, an age grouping, a breed or type classification, a weight chart, and a record of the general physical condition of the animal during the period of observation. Animals that did not remain well and in good physical condition were excluded from this series but were used as a check against the others and to supply data on the health of animals kept under the conditions mentioned above. Groups of five to ten animals were killed at intervals of 2 to 3 weeks. As a rule, there were two groups for each month; the first group was killed near the middle of the month and the second at the end of the month, thus giving a total of a least ten rabbits and usually as many as fifteen or twenty for each month with the exception of July, 1922, and August, 1922 and 1923. It was impracticable to maintain routine observations during these months but some data are available for July, 1922.

The method of conducting the final examination was as follows: Animals received no food on the day they were killed; they were weighed, then anesthetized with ether and bled from the inferior vena cava while the heart was still beating. More recently, killing by air embolism has been substituted for the ether as a quicker and less disturbing procedure. Autopsy was performed immediately. The postmortem examination included two sets of observations: first, the noting of anatomical malformations or defects and of all gross evidences of disease, whether in the form of active or healed lesions, and second, the making of weight determinations on certain organs and tissues. These observations were later supplemented by microscopic examination of tissues from a number of organs.[1] No animal was excluded from the series on account of postmortem evidence of the presence of disease, but the results of the postmortem examination were used as the basis for classifying animals in a study of the relation of various pathological conditions to organ weight, the results of which will be reported in another paper.

The list of organs weighed has been changed several times since the investigations began so that there is considerable variation in the number of animals for which weights of different organs are available. The complete list of organs weighed is as follows: the heart, liver, kidneys, spleen, the gastrointestinal mass (including stomach, intestine, mesentery, omentum, and gastrointestinal contents), the brain, the testicles, the thymus, the thyroid (with internal parathyroids), external parathyroids (usually two in the neck region), suprarenals, hypophysis, the pineal gland with its stalk and the associated plexus of vessels, the popliteal, posterior axillary, and the deep cervical lymph nodes of both sides, and the main mesenteric mass of lymph nodes. The number of animals on which weights are available in each case is given in Table I.

The urine in the bladder was measured as a means of making corrections for body weight.

In making the weight determinations the work was so distributed that removal of the organs from the body, the preparation for weighing, and the weighing itself proceeded parallel and uniformly with little or no delay at any stage of the process. All organs were carefully prepared for weighing by trimming and removal of extraneous tissues. The cardiac vessels were cut on a level with the outlines of the auricles; the chambers were opened and all blood removed before weighing. The brain was cut anteriorly at the junction of the frontal and olfactory lobes and posteriorly at the lower margin of the fourth ventricle or on a level with the ventral margin of the foramen magnum so that the weights of the brain include the cerebrum, cerebellum, pons, and medulla but not the olfactory lobes. Weights of the testicle include the globus major but other parts of the epididymis were removed. No effort was made to separate the glandular tissue of the thymus from the fat contained within the capsule so that the weights for this organ represent the weight of the thymic mass rather than of the thymus itself. Weights of the

[1] This phase of the work is as yet incomplete.

hypophysis represent only the anterior, middle, and posterior lobes, but in the case of the pineal gland the entire stalk together with the associated plexus of vessels is included so that only a small part of the weights recorded represent glandular tissue. In order to avoid drying after removal from the body, the small organs were placed in Petri dishes and kept moist with physiological salt solution. Before weighing, the excess fluid was removed by absorption on filter paper.

The gastrointestinal mass and the liver were weighed on balances sensitive to 0.1 gm.; the heart, kidneys, brain, testicles, and mesenteric lymph nodes were weighed on balances sensitive to 0.01 gm.; while all other organs were weighed on balances sensitive to 0.001 gm.

The data recorded in this paper include the results of a primary analysis of actual weights and of weights per kilo of net body weight; *i.e.*, the gross weight minus the weight of the gastrointestinal mass as defined above and of urine.

The distribution curves given in Text-figs. 1 to 3 are based on the use of a group interval of approximately one-tenth of the average weight of the organ or the weight per kilo of body weight. The interval used in a given instance was determined by convenience but in plotting the distribution of actual weights and of weights per kilo of body weight for a given organ a constant ratio of 2:1 was maintained. The curves obtained by plotting the actual results were smoothed by the formula $\frac{a+2b+c}{4}$. The smoothed curve is graphed as the broken line in Text-figs. 1 to 3.

In some instances the curves are not extended to the upper limits of weight, as given in Table I, but the weights omitted are few and scattered. The limit to which a curve should be extended is indicated by a figure placed at the termination of the curve. Curves not marked in this way are complete.

The figures as given include all data irrespective of whether an organ was normal or pathological. It seemed best to present the material in this form before attempting to make corrections for disease or any other factor that might give rise to variations in weight. These subjects will be dealt with in subsequent papers.

RESULTS.

The results obtained from this series of weight determinations are presented in condensed form in Table I and Text-figs. 1 to 3.

DISCUSSION AND CONCLUSIONS.

In discussing the experiments reported, it should be pointed out at once that the results obtained in this instance are neither strictly comparable with those given in other reports dealing with organ weights of normal rabbits nor do they represent results that

might be expected from the examination of a group of animals where an effort is made to maintain uniformity of material and experimental conditions. One of the purposes of this investigation was to define the possible as well as the probable limits of variation in the weight of certain organs of rabbits comparable in all respects to those used in the conduct of other experiments. This has necessitated the use of animals of various ages and breeds and the extension of the observations over a long period of time. Finally, in analyzing the results, the problem has been approached first from the standpoint of the individual animal rather than the several groups which compose the series. All of these conditions tend to emphasize the element of variation which, at present, is the aspect of the subject in which we are most interested.

With these facts in mind, the first point to be considered is the reliability of the data as an index of existing conditions. There are only a few instances in which this question need be discussed. The weights that are open to question are those of the thymus, the parathyroids, the pineal gland, and the superficial and mesenteric lymph nodes. It is obvious that the weights of these organs cannot be determined with the same degree of accuracy as the weights of the other organs.

The chief source of error in nearly all instances is in the presence of variable amounts of fat or areolar tissue which cannot be removed satisfactorily in the preparation of the organs for weighing. This applies especially to the thymus and the lymph nodes, but a similar condition exists in the case of the pineal gland. The parathyroids present difficulties of another kind. Only the external parathyroids are available for weight determinations. As a rule, there are two of these but occasionally only one can be found, or there may be three—rarely four. There are other masses of parathyroid tissue in the thyroid and the thymus and not infrequently the external parathyroids themselves contain small masses of thymic tissue. At all events, there is no way of determining whether the external parathyroids represent a relatively constant part of the parathyroid tissue and a sufficiently large part to serve as an index of the amount of such tissue present except by making a large series of weight determinations.

TABLE I.

Summary of Numerical Values Obtained from Determinations of Body and Organ Weights Made on 350 Male Rabbits of Various Ages and Breeds between January 1, 1922, and July 1, 1924.

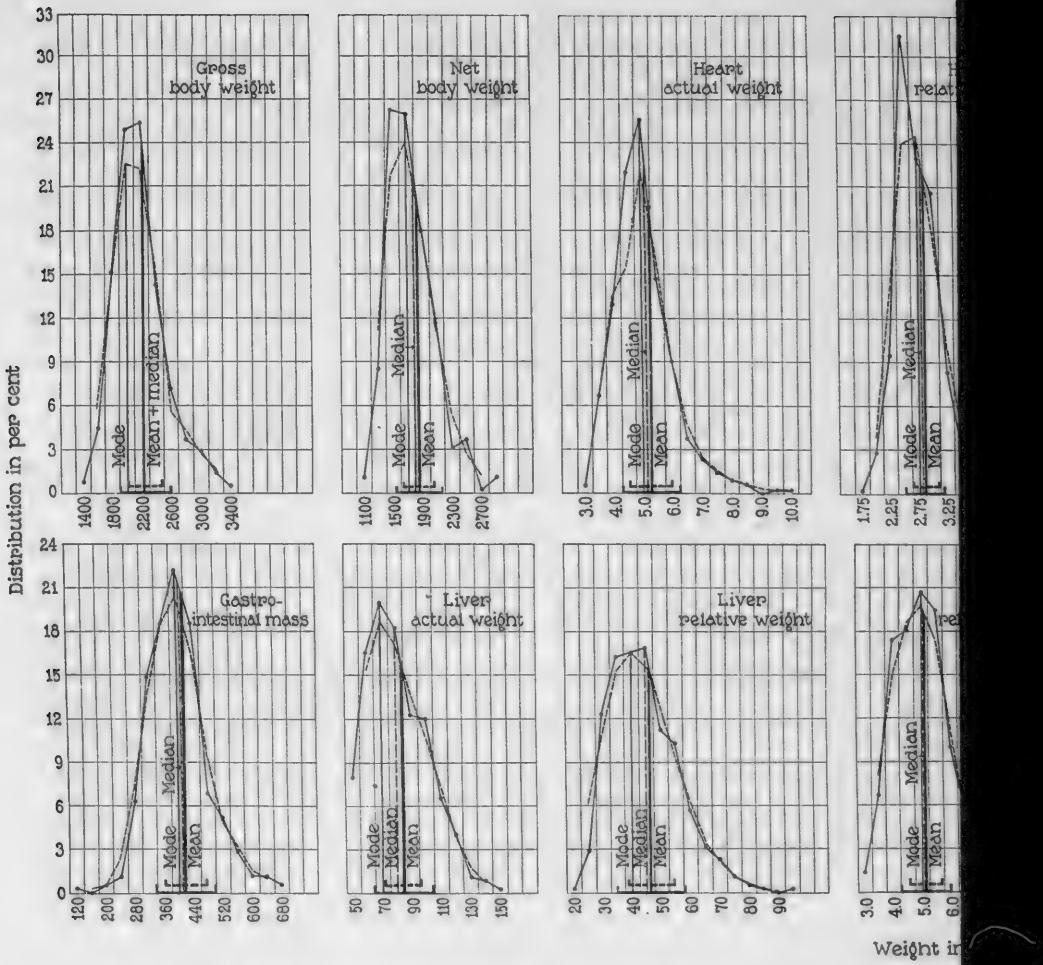
The upper row of figures in each couplet gives the values for actual weights while the lower figures are for weights per kilo of net body weight.

Organ.	No. of animals.	Arithmetical mean.	Median.	Mode.	Minimum.	Maximum	Standard deviation.	Probable error.	Coefficient of variation.
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	± *gm.*	± *gm.*	*per cent*
Gross body weight	350	2,240.30	2,225	2,000	1,400	3,500	352.62	237.84	15.74
Gastrointestinal mass	350	416.35	410	400	115	710	80.85	54.53	19.42
Net body weight	350	1,848.50	1,800	1,700	1,150	3,010	323.27	218.04	17.49
Heart	348	5.26	5.12	5.00	3.20	10.18	0.96	0.65	18.23
		2.85	2.81	2.75	1.95	4.42	0.35	0.24	12.32
Liver	350	84.30	80.00	70.00	50.00	150.00	19.72	13.30	23.39
		46.46	44.90	40.00	23.20	97.40	11.84	7.99	25.48
Kidneys	348	12.70	12.50	12.00	7.15	22.73	2.07	1.40	16.30
		6.97	6.84	6.50	3.45	17.28	1.26	0.85	18.08
Spleen	347	0.99	0.90	0.70	0.08	3.20	0.436	0.294	44.04
		0.531	0.49	0.45	0.035	1.714	0.214	0.144	40.30
Thymus	349	2.21	2.13	1.75	0.45	5.90	0.848	0.572	38.37
		1.23	1.147	1.00	0.334	2.72	0.451	0.304	36.64
Testicles	290	4.86	4.85	4.50	0.81	9.12	1.63	1.10	33.54
		2.61	2.62	2.50	0.47	4.93	0.70	0.47	26.90

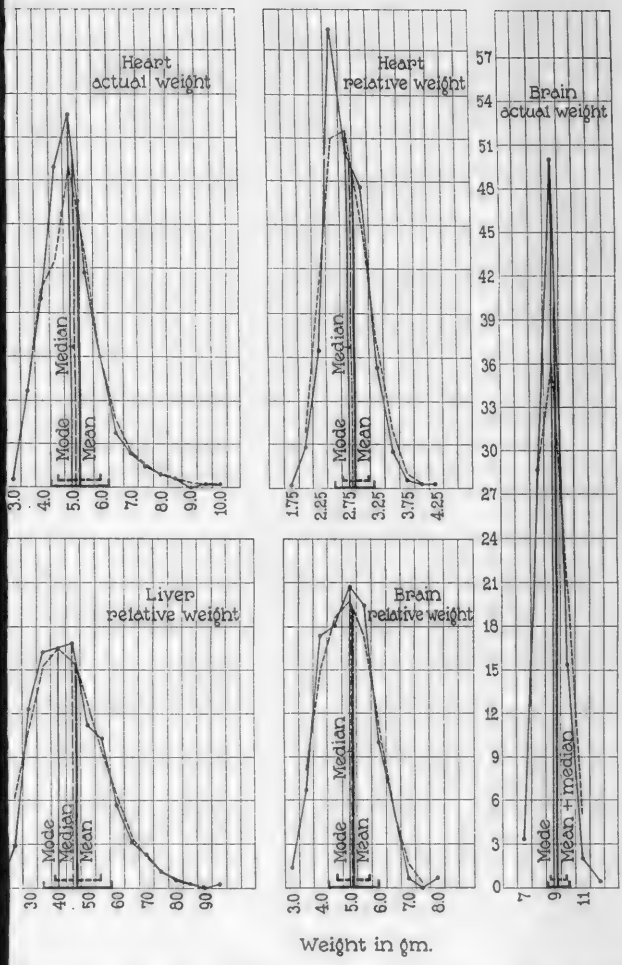
Brain	150	9.31	9.24	9.00	7.42	12.03	0.78	0.526	8.37
		5.113	5.06	5.00	3.33	8.16	0.87	0.58	16.92
		mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	
Thyroid	349	230.48	185.00	160.00	85.00	1,570.00	162.09	109.33	70.33
		123.24	97.50	90.00	50.40	730.00	75.7	51.06	61.47
Parathyroids	349	12.56	12.00	10.00	2.00	35.00	4.86	3.28	38.69
		6.92	6.31	5.00	1.09	21.75	2.78	1.87	40.13
Hypophysis	348	28.00	28.00	26.00	15.00	44.00	5.17	3.49	18.46
		15.40	15.30	14.50	7.28	29.40	3.10	2.09	20.27
Suprarenals	349	380.12	358.00	260.00	115.00	1,050.00	155.01	104.55	40.78
		208.80	196.70	170.00	80.40	530.00	75.03	50.61	35.93
Pineal gland	348	16.09	15.00	14.00	5.00	30.00	4.29	2.89	26.66
		8.85	8.68	8.00	2.73	18.73	2.45	1.65	27.68
Popliteal lymph nodes	242	255.39	245.00	210.00	85.00	720.00	82.41	55.59	32.27
		135.73	136.10	150.00	52.50	382.00	48.73	32.87	35.90
Axillary " "	242	175.85	175.00	160.00	40.00	400.00	59.75	40.30	33.98
		97.22	91.00	80.00	18.65	242.00	37.79	25.49	38.87
Deep cervical lymph nodes	124	155.79	140.00	100.00	35.00	405.00	73.20	49.38	46.98
		82.07	72.40	60.00	20.52	211.00	37.12	25.04	45.23
		gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
Mesenteric " "	133	3.488	3.32	3.00	1.34	9.19	1.31	0.88	37.53
		1.828	1.763	1.70	0.72	3.71	0.642	0.43	35.12

At the beginning of our investigations, it was feared that the error attributable to the conditions mentioned above would be so large that it would completely overshadow any variations in the weights of glandular tissue and thus invalidate the results of weight determinations on this group of organs. Experience has shown, however, that this is not entirely true. This conclusion is indicated by a comparison of the results obtained for these organs with the results obtained for organs of a similar nature on which accurate weight determinations can be made. By reference to Table I, it will be seen that the values given for the parathyroid and the pineal gland are of the same general order as those given for the thyroid or the suprarenals and that the values for the thymus and lymph nodes are comparable to those for the spleen and the deep cervical lymph nodes. The dependence that may be placed upon weights of the thymus, the parathyroids, the pineal gland, and lymph nodes will become more apparent when we consider these organs from the standpoint of the various groups of animals composing the series. Meantime, it may be said that, while we regard the weights of these organs as possessing some significance, we recognize the existence of an unavoidable error, the usual effect of which is to increase weights and to minimize variations in the actual mass of glandular tissue. This error is most disturbing in the case of the pineal gland and the parathyroids due to their very small size.

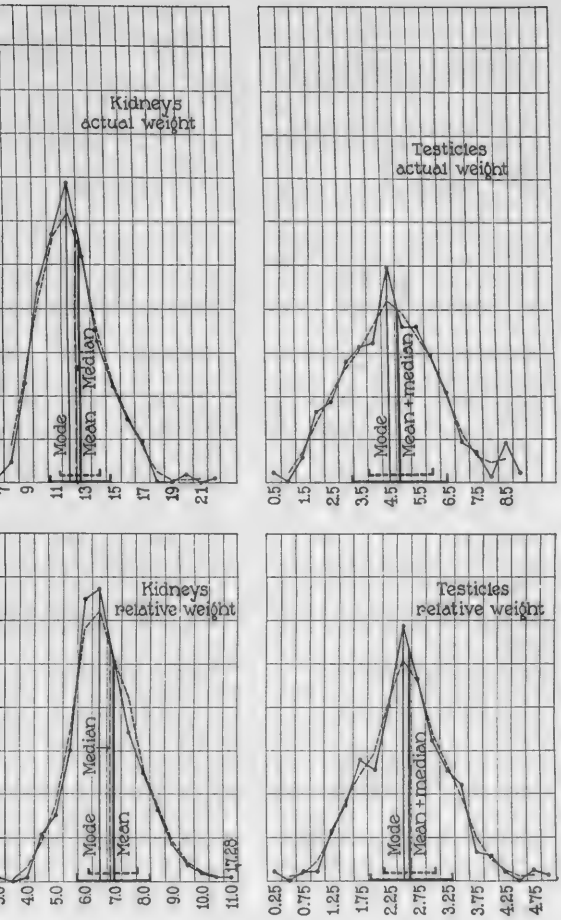
As regards the significance of the results in general, it is of interest to note that, in cases where comparisons can be made, values obtained for the rabbit appear to be of essentially the same order as those given for man. With the coefficient of variation as a basis for comparison, the values given by Pearl (2) for man and those obtained by us for the rabbit are as follows:



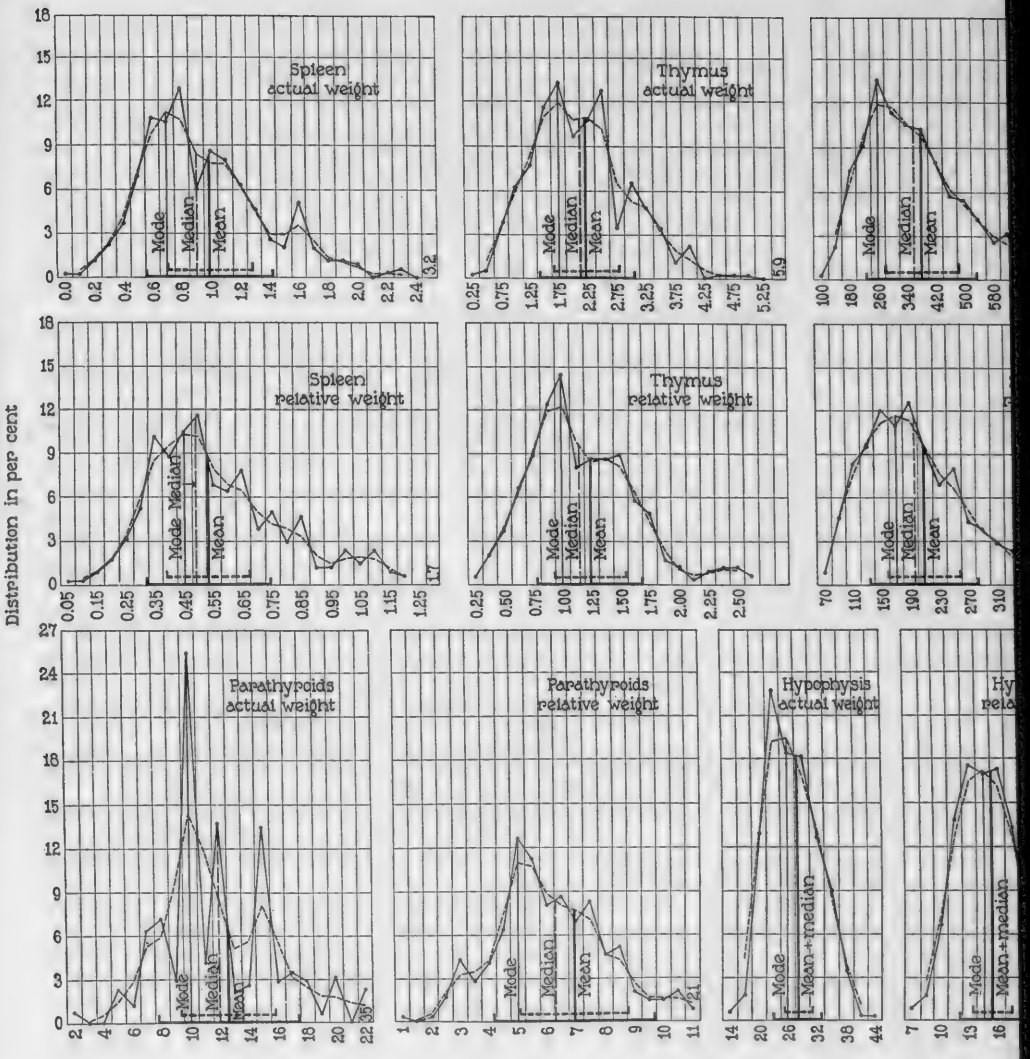
TEXT-FIG. 1. Curves of actual and relative weight distribution in per cent. The brackets at th



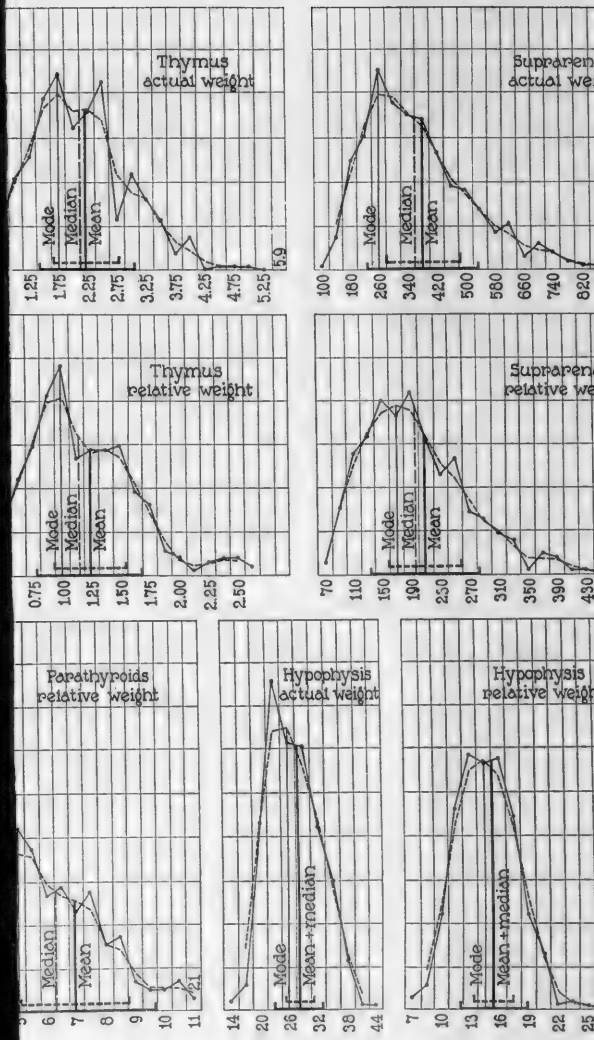
tribution in per cent. The brackets at the bottom of the curves mark



the limits of the standard deviation and the probable error.

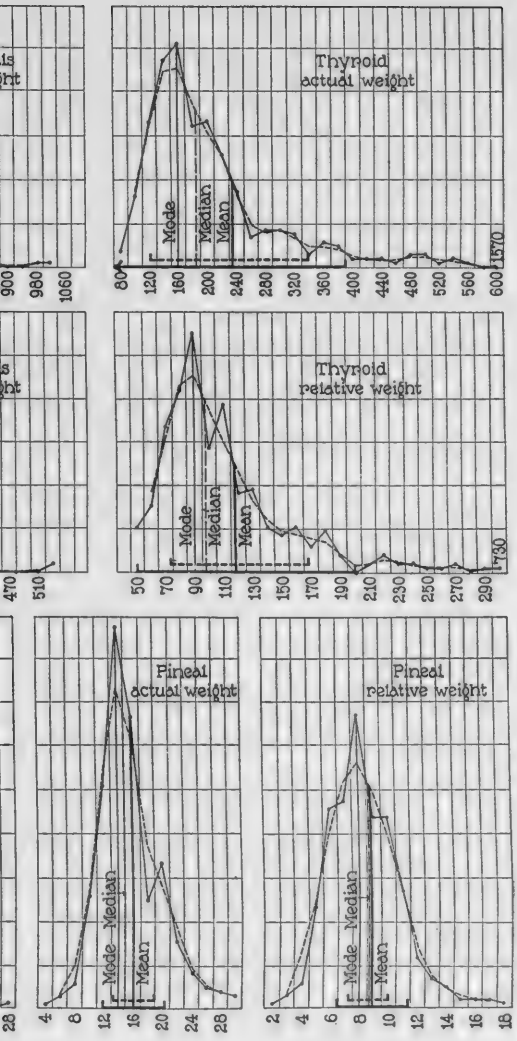


TEXT-FIG. 2. Curves of actual and relative weight distribution in per cent. The brackets at the
and the probable error.



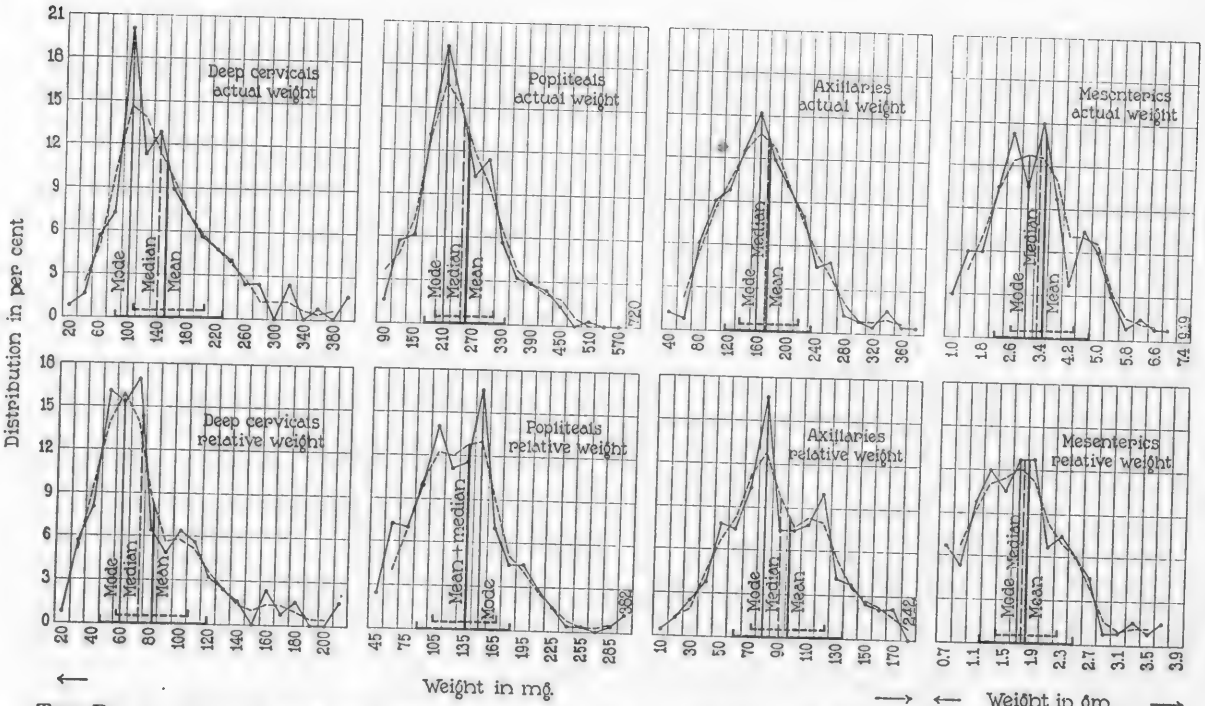
Weight in gm. for spleen and thymus, weight in mg. for othe

weight distribution in per cent. The brackets at the bottom



organs

f the curves mark the limits of the standard deviation



TEXT-FIG. 3. Curves of actual and relative weight distribution in per cent. The brackets at the bottom of the curves mark the limits of the standard deviation and the probable error.

	Man.	Rabbit.	
Weight of body.			
Bavarians	21.32	15.74 (gross).	All breeds.
English	10.37	17.49 (net).	" "
Weight of heart.			
Healthy	17.71	18.23	Normal and pathological.
Gen. Hosp. Pop.	32.39		
Weight of liver.			
Healthy	14.80	23.39	" " "
Gen. Hosp. Pop.	21.12		
Weight of kidneys.			
Healthy	16.80	16.30	" " "
Gen. Hosp. Pop.	24.63		
Weight of spleen.			
Healthy	38.21	44.04	" " "
Gen. Hosp. Pop.	50.58		
Weight of brain.			
Various races (males)	7.592	8.37	All breeds.
	7.809		
	8.096		
	8.118		
	9.160		

In most instances the agreement between the two sets of figures is surprisingly close, while in no case is there a serious disagreement.

Referring briefly to some of the more important features of the results, the first thing to which attention should be directed is the variation in organ weights. An examination of the data contained in Table I and the distribution curves will show that the weights of nearly all organs are subject to wide and frequent variations whether we consider the actual weight or the weight per unit of body weight. The notable exceptions to this rule are to be found in the case of the brain, the heart, the kidneys, and the hypophysis, which show coefficients of variation less than or of the same general order of magnitude as those for the gross body weight, the net body weight, or the gastrointestinal mass. At the other extreme are found such organs as the thyroid, the deep cervical lymph nodes, the spleen, the suprarenals, and the parathyroids with coefficients of from two to four times the coefficients for body weight.

The exact order of variation depends somewhat upon whether one considers the actual weight of the organ or the weight per unit of body weight. This may be shown by arranging the organs in parallel columns according to the magnitude of the coefficient of variation.

Gross body weight	15.74
Net body weight	17.49
Gastrointestinal mass	19.42

Actual weight.		Weight per kilo.	
Brain	8.37	Heart	12.32
Kidneys	16.30	Brain	16.92
Heart	18.23	Kidneys	18.08
Hypophysis	18.46	Hypophysis	20.27
Liver	23.39	Liver	25.48
Pineal gland	26.66	Testicles	26.90
Popliteal lymph nodes	32.27	Pineal gland	27.68
Testicles	33.54	Mesenteric lymph nodes.	35.12
Axillary lymph nodes	33.98	Popliteal " "	35.90
Mesenteric " "	37.53	Suprarenals	35.93
Thymus	38.37	Thymus	36.64
Parathyroids	38.69	Axillary lymph nodes	38.87
Suprarenals	40.78	Parathyroids	40.13
Spleen	44.04	Spleen	40.30
Deep cervical lymph nodes	46.98	Deep cervical lymph nodes	45.23
Thyroid	70.33	Thyroid	61.47

This form of arrangement does not disturb the general order of variability but merely the relative position of different organs. That is, the same organs occupy the first five positions in both columns but their relative positions change. This situation obtains until we reach the three lowest positions; here the order is the same in both cases.

Essentially the same result is obtained if we employ any other method of comparison. It is possible to say, therefore, that among the organs studied the brain, the heart, the kidneys, the hypophysis, and the liver give the most constant values, while the weights of such organs as the thyroid and the spleen and the organs occupying a position between these two groups give values that are subject to much wider variation.

The comparison of coefficients of variation for actual and relative weights brings out another important point in mass relationships; namely, the degree of correlation that exists between the weight of an

organ and body weight. As yet, the correlation coefficients have not been calculated as the material on which this study is based did not seem to be sufficient to warrant the use of such an exact method, but a comparison of the figures given in Table I and of distribution curves would indicate that the degree to which the weight of an organ is dependent upon body weight is itself a matter of considerable variation.

In the majority of cases, it would appear that the weights of organs are closely related to body weight, but there are a few instances in which the weight of the organ seems to be independent of body weight within certain limits. The most striking example of this is found in the case of the brain which shows a coefficient of variation for actual weight which is much smaller than that for the relative weight. There are other organs that may fall into the same class of relative independence such as the hypophysis and the kidneys. There are still other organs that show comparatively little difference between the constancy of the values obtained on the basis of actual and relative weights, but where the differences are so small and the coefficients large it is obvious that a larger amount of material will be required to determine the exact relationships. Meantime, it is worthy of note that organs occupying an intracranial position (the brain and the hypophysis) show an apparent tendency to conform to certain limits of weight independent of body weight. We do not mean to imply that the weights of these organs are fixed. On the contrary, it will be shown in a subsequent paper that, under certain circumstances, they vary as do the weights of other organs.

Another feature of the results to which reference may be made is the frequency with which organs of a given weight may be encountered and the probable direction of variation from the mean, mode, or median, as the case may be. These points are brought out in the distribution curves (Text-figs. 1 to 3) which have been grouped with a view to indicating the conditions that obtain in different classes of organs or tissues such as the parenchymatous organs, the organs of internal secretion, and the lymphoid tissues, the curves for which are given in the order mentioned.

It will be seen that a number of the distribution curves are of the same general form as those for body weight. The curves for the heart,

the liver, the kidneys, the brain, the hypophysis, the pineal gland, and the gastrointestinal mass are almost symmetrical; they show a relatively narrow range of weight distribution with a rapid rate of increase and decrease in the percentage of organs whose weight is close to the mean or mode and little or no tendency to the prolongation of the curves at either extreme. The curve for the testicles is also fairly symmetrical but shows a wider range of variation.

The distribution frequencies of other organs are much more irregular. The thyroid, the suprarenals, and some of the lymph nodes show a tendency to follow the same general form of weight distribution as the organs mentioned above but the range of weight is greater, the curves are irregular, and decidedly skewed, due chiefly to the occurrence of varying numbers of organs whose weights lie well above the mean. These conditions not only extend the zone of high frequency distribution but increase the probable error in estimations of the frequency of occurrence of organs of a given weight.

A third type of distribution is illustrated by such organs as the spleen and thymus. The feature of special interest in these curves is the presence of a wide zone of high frequency distribution within which there is comparatively little difference in the frequency with which organs of given weights may occur. Some of these curves are almost symmetrical while others are decidedly skewed.

The relative positions of the mean, the mode, and the median conform, in general, to theoretical expectations in nearly all cases. The only instance in which the mean and the median occupy a lower position than the mode is in the curve for the weight of the popliteal lymph nodes per kilo of body weight. This is probably an apparent rather than a real displacement which would not occur in the case of a fitted curve.

It seems unwise to carry the statistical analysis of the material further at the present time. The values as here given are affected by a number of variables whose influence must be considered before an attempt may be made to establish more exact values or to apply the results to any specific problem, except for purposes of orientation.

In the next paper, we will consider effects of pathological conditions of spontaneous origin which were not apparent clinically but were demonstrable by postmortem examination.

SUMMARY.

In November, 1921, a systematic study of normal rabbits was undertaken as a part of a more general investigation dealing with the subject of the animal organism in relation to disease. The present paper on organ weights is based on results obtained from a study of 350 male rabbits killed and examined between January 1, 1922, and July 1, 1924. Methods of conducting the experiments are described and the results are summarized in the form of a table and a series of text-figures.

The organs studied were the heart, liver, kidneys, spleen, thymus, testicles, brain, thyroid, parathyroids, suprarenals, hypophysis, pineal gland, and representative groups of lymph nodes.

The results recorded include maximum, minimum, and average weights, the median, the mode, the standard deviation, the probable error, the coefficient of variation, and the percentage distribution of organs of different weights.

No final conclusions are drawn but it is pointed out that the results obtained are comparable to those that have been reported from similar studies of organ weight in man. Attention is also directed to the tendency to the occurrence of wide variations in the weights of nearly all organs, and to an apparent difference in the degree of correlation that exists between organ weight and body weight in the case of certain organs. In this connection it is pointed out that within certain limits the weight of the brain, in particular, and of other organs to a lesser degree appears to be independent of body weight.

The results recorded in this paper are regarded as representing approximate values which are affected by numerous conditions for which correction should be made. These conditions will be considered in subsequent papers.

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2. Pearl, R., *Biometrika*, 1905, iv, 13; Medical biometry and statistics, Philadelphia, 1923, 276.

DRAINAGE OF THE THORACIC DUCT IN EXPERIMENTAL PERITONITIS.

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Subperitoneal capillaries and lymphatics are the two known channels of absorption from the peritoneal cavity. The mechanism of the route first mentioned has been clarified largely by the work of Starling and Tubby[1] but that of the lymphatic path still remains obscure.

Danielson,[2] 1907, found that colloids are taken up by the lymph channels and transported through the thoracic duct while crystalloids are absorbed by the capillaries. Since bacteria belong to the class of colloids it has been assumed that they are absorbed by the lymphatics and carried along the thoracic duct into the general circulation. Even before the work of Danielson, Noetzel,[3] 1898, ligated the thoracic duct in animals and attempted to produce peritonitis by intraperitoneal injections of virulent staphylococci. He found that the animals with the ligated thoracic duct, as well as the controls with the intact duct, survived the intraperitoneal injection of the bacteria. This work was not conclusive because Noetzel failed to produce fatal peritonitis in his animals. Costain,[4] 1922, tied the appendix and the mesoappendix in dogs, ligated the thoracic duct, and produced a fistula by puncturing the duct below the ligature. He reported the recovery of some of the animals from what he assumed to be an otherwise fatal peritonitis resulting from the ligation of the appendix and the mesoappendix.

The study here presented was undertaken to determine (1) whether or not an animal with an experimentally produced and ordinarily fatal peritonitis can be kept alive by the drainage of the thoracic

* Fellow in Medicine of the National Research Council.

[1] Starling, E. H., and Tubby, A. H., *J. Physiol.*, 1894, xvi, 140.

[2] Danielson, W., *Beitr. klin. Chir.*, 1907, liv, 458.

[3] Noetzel, W., *Arch. klin. Chir.*, 1898, lvii, 311.

[4] Costain, W. A., *Canad. Med. Assn. J.*, 1922, xii, 789.

duct, and (2) whether or not bacteria are transported through the thoracic duct into the general circulation. To accomplish these ends it was necessary to establish a satisfactory method for a continuous drainage of the thoracic duct and to be able to produce a regularly fatal peritonitis in dogs, which were the animals of choice.

Drainage of the Thoracic Duct.

Drainage of that part of the thoracic duct within the chest was considered, but discarded because in addition to the objections to direct drainage mentioned below, it exposed the animal to greater operative hazards. Ligation of the thoracic duct as practiced by Noetzel and later by Costain seemed unsatisfactory because of the inability to collect uncontaminated lymph for bacterial examination or to obtain information upon the constancy or amount of drainage, or the fate of the lymph owing to the circumstance that it drained into the wound of the tissues of the neck. A more satisfactory method is to drain the thoracic duct either directly or through the veins into which it enters. Jappelli[5] in studying lymph pressure inserted a cannula directly into the thoracic duct near its entrance into the external jugular vein. This method was tried. Several difficulties were encountered in this procedure. The lymph clotted very rapidly, and it was found impossible to change the tubes or to cleanse them without mutilating the duct. In a prolonged experiment where an uninterrupted flow of lymph is essential such a procedure was found inadequate.

Jappelli[6] and Lee[7] in further studies on lymph pressure ligated the innominate, subclavian, and internal jugular veins and obtained lymph pressure readings by connecting a manometer with the external jugular vein. This method, somewhat modified, is the one I have employed to secure a continuous flow of lymph from the thoracic duct. Since little information could be gathered from the literature concerning the effect of thoracic duct drainage upon the general condition of the dog, observations were made on the "draining animals" and for some period of time after the drainage had ceased.

[5] Jappelli, G., *Arch. sc. biol.*, 1920, i, 193.
[6] Jappelli, G., *Zentr. Physiol.*, 1905–06, xix, 161.
[7] Lee, F. C., *Am. J. Physiol.*, 1923–24, lxvii, 498.

Technique of Indirect Drainage.

The animals were given 1 pint of milk 2 hours prior to the operation in order to facilitate the identification of the duct. All other food except water was withdrawn about 12 hours before the operation. Ether anesthesia was used. The animals were kept under ether 1½ hours at the most, the actual operation occupying from 30 to 45 minutes. A curved incision was made with the convexity directed medially from the middle of the medial border of the sternocleidomastoid muscle to the junction of the outer and middle thirds of the clavicle. The external jugular was dissected out to its junction with the internal jugular, all tributaries being ligated. At this juncture the thoracic duct could be recognized as it enters at the junction of the external and internal jugulars along the posterior and lateral aspect of the former. With the duct identified, the internal jugular was ligated and another ligature placed immediately beyond the duct in front of the subclavian vein. Occasionally, the duct joined the external jugular at the point of entrance of the subclavian, which necessitated the ligation of the innominate and subclavian veins individually. A small buttonhole incision corresponding to the diameter of the lumen of the external jugular was made in the skin. The free end of the cardiac portion of the external jugular was sutured to the buttonhole skin incision so that a fistula with a continuous endothelial lining was established, which reduced clotting appreciably. The insertion of a short cannula improved the drainage. The removal of the cannula for cleansing was effected with ease.

The indirect lymph drainage was established in four dogs. One animal drained for 5 days, the other three for 2 days. One died on the 3rd day of pneumonia. The cessation of the external drainage of lymph was due to the necrosis of the vein segment sutured to the skin. The animals did not show any untoward symptoms during the period of drainage nor after the flow of lymph had ceased.

Induction of Peritonitis.

Noetzel injected virulent bacteria into the peritoneal cavity; Costain tied the appendix and its blood supply to induce a fatal peritonitis. I tried both methods and failed to secure fatal peritonitis. Recently, Lehman and Copher[8] repeated Costain's experiments by ligation of the appendix and the mesoappendix on fourteen animals and reported four recoveries.

Benians[9] produced fatal peritonitis in rabbits by the combined intraperitoneal injection of gum tragacanth and *B. coli*. This procedure was tested and it was found that 20 cc. of a 5 per cent suspension of gum tragacanth in distilled water

[8] Lehman, E. P., and Copher, G. H., *Arch. Surg.*, 1925, x, 742.

[9] Benians, T. H. C., *Brit. J. Exp. Path.*, 1924, v, 123.

and 20 cc. of a 24 hour broth culture of *B. coli* (isolated from a fatal case of human peritonitis and passed through a guinea pig, two cats, and two dogs) were the minimum quantities necessary to produce a fatal peritonitis in a dog weighing 10 kilos, death ensuing from 16 to 26 hours after the injection.

Six animals were injected with the quantities given above of gum tragacanth and *B. coli* and they all died from 16 to 24 hours after the injection. The autopsies upon the animals revealed an intense peritonitis with hyperemia of the visceral and parietal peritoneum, omentum, and diaphragm, a fibrinous exudate, and from 150 to 250 cc. of a viscid, red fluid containing erythrocytes, leucocytes, and *B. coli*. The abdominal organs showed cloudy swelling; cultures of the heart's blood were negative. Control animals that were injected with gum tragacanth alone and with a broth culture of *B. coli* alone did not succumb.

Induction of Peritonitis and Drainage of the Thoracic Duct.

Peritonitis was induced by the injection of gum tragacanth and *Bacillus coli* as described above. The thoracic duct was drained by the method already given. In two animals blood cultures from the femoral vein were taken simultaneously with lymph cultures. For each animal that was drained a control was used.

Four animals whose ducts were drained died from $7\frac{1}{2}$ to 10 hours after the intraperitoneal injection of the gum tragacanth and *Bacillus coli*. From 1 to $6\frac{1}{2}$ hours were allowed to elapse after the completion of the operation before the intraperitoneal injections were made. The peritoneal cavity in these animals revealed an intense peritonitis but with very little free fluid. The blood cultures taken from the femoral vein in two animals were negative for the bacteria injected into the peritoneal cavity. Neither smears nor cultures of the lymph taken at frequent intervals, except two cultures in one dog, revealed *Bacillus coli*. Of the four controls two survived. The two controls that succumbed died 17 hours after the intraperitoneal injections. Both revealed practically the same peritoneal conditions as the drained animals except for the presence of a free peritoneal fluid in quantities of 250 cc.

DISCUSSION.

The drainage of the thoracic duct of itself did not lead to any apparent ill effects. There was no appreciable loss in weight or strength. The flow of lymph varied from 9 to 15 cc. per hour, the

quantity depending upon the nourishment received and the activity of the animal. The character of the lymph varied from a viscid milky white to a thin, red-tinged or clear fluid.

The resistance of dogs to peritoneal infections is remarkable. In some preliminary experiments the ligation of the appendix and the mesoappendix resulted in a peritonitis which was usually localized and not necessarily fatal. The introduction of highly pathogenic microorganisms into the peritoneal cavity sometimes induced a mild peritonitis but did not produce fatal results. The reason for a fatal termination when employing a mixture of gum tragacanth and microorganisms is, according to Benians, the protective covering that the gum offers the bacteria. He found that the bacteria embedded in gum preserved their vitality for several weeks. The failure of two of my controls to succumb to the tragacanth and *Bacillus coli* mixture may be due to the unusually slight virulence of the bacteria or to an individual resistance of the animals.

Drainage of the thoracic duct did not save from peritonitis otherwise fatal. In fact the draining animals lived for a smaller number of hours than the controls. That, however, may be ascribed to the operative trauma and etherization. Lehman and Copher reached such a conclusion in their experiments in which they repeated the methods employed by Costain.

The work was undertaken on the assumption that bacteria or their products enter the lymphatics and are transmitted through the thoracic duct into the blood. It was further assumed that death in peritonitis is due to the invasion of the circulation by bacteria or their products. The latter assumption has not yet been proved. The finding of bacteria in the blood of animals with peritonitis by some investigators and the failure to do so by others may be due to differences in the species of animals employed. Observations were made, which will be published elsewhere, on rabbits, whose peritoneal resistance to infections is comparatively low; and bacteria were found in their blood while in the case of cats and dogs, whose resistance is higher, bacteria were not recovered.

The recent work of Bolton,[10] 1921, on peritoneal absorption of

[10] Bolton, C., *J. Path. and Bact.*, 1921, xxiv, 429.

dyes throws some doubt on whether the thoracic duct is of any consequence in draining the peritoneal cavity. He arrives at the conclusion that the peritoneal cavity is drained primarily by the right lymphatic duct and only to a small extent by the thoracic duct. He further concludes that colloidal dyes and bacterial products of not too great molecular complexity may be absorbed directly into the blood from the peritoneal cavity while colloids of greater molecular weight are taken up by the lymphatics.

CONCLUSIONS.

1. When care is taken to produce a peritonitis, which in itself is almost uniformly fatal, drainage of the thoracic duct in dogs has no favorable influence upon the course or result of the peritonitis.

2. Under the conditions of my experiments the thoracic duct did not serve in any important way as a drainage tract for bacterial contamination of the peritoneal cavity.

I wish to acknowledge by indebtness to Dr. Harry Goldblatt for his valuable aid in this work.

THE RELATION OF HYPERGLYCEMIA TO THE RELATIVE BLOOD VOLUME, CHLORINE CONCENTRATION, AND CHLORINE DISTRIBUTION IN THE BLOOD OF DOGS.

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(Received for publication, April 7, 1925.)

Elsewhere I have discussed the relation of hyperglycemia to the mobilization and distribution of water and chlorine in the blood of diabetic patients (1). On the basis of qualitative changes in blood concentration these patients were found to be separable into two general groups. The first group comprised those patients whose response to hyperglycemia was a concentration of the circulating blood, as evidenced by a diminished relative volume of serum, high erythrocyte counts, increased viscosity of the blood to gross inspection, and frequently an increased concentration of chlorine within the erythrocytes. Reduction of the hyperglycemia permitted a reversal of these abnormalities. The second group consisted of all patients whose response to hyperglycemia was a blood dilution: increased relative volume of serum, subnormal erythrocyte counts, no increase in viscosity of the blood grossly, and extremely variable corpuscular chlorine concentrations. Reduction of the hyperglycemia also caused a reversal of these abnormalities in this group.

All juvenile diabetics and all cases of diabetes in young adults were to be found only in the first group. In addition, certain elderly diabetics, who either had suffered severe acute exacerbations or whose blood was examined during the terminal phase of the disease, were also found in the first group. With these exceptions, diabetic patients with recognizable arteriosclerosis were found only in the second group. It is evident then that reduction of a hyperglycemia (or the action

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of insulin) may produce *either* an increase or a decrease in the concentration and viscosity of the blood, the result in any given case depending upon the antecedent conditions. This fact has received exceedingly scant attention from workers in this field, probably because of the extreme difficulty encountered in attempts to reproduce in experimental animals the conditions that are found in the young diabetic patient.

Hyperglycemia and some of the related and associated phenomena have been studied in the blood of dogs. It was desired (*a*) to produce, if possible, a dehydration of the blood by means of glucose alone, (*b*) to corroborate the findings previously observed in patients, and (*c*) to investigate further the relation of chlorine concentration and its distribution to hyperglycemia.

Dehydration of the blood by means of glucose alone was not achieved. In spite of large quantities given rapidly—sometimes more than 10 gm. per kilo body weight per hour—and despite enormous diuresis, there was never a reduction of the relative volume of serum. The amount of water injected with the glucose (50 per cent solution) was insufficient in itself to prevent dehydration. In one instance the total amount injected represented less than one-tenth of the loss by diuresis. The longest injection period was $4\frac{1}{2}$ hours. It is highly probable that the tissue water supply of the normal dog is great enough to prevent dehydration of the blood by glucose over short periods of time. If administration of large amounts were continued long enough, it is conceivable that the constant diuresis must diminish the total volume of body water so that ultimately the tissue supply would become exhausted and blood concentration would result. One such attempt resulted in the death of the dog, and even then no evidence of blood concentration was obtained. Hence it was not found possible to reproduce experimentally the conditions that existed in the young diabetic patients of the first group at the time they were admitted to the hospital.

Studies on Serum.

When glucose is administered to the unanesthetized dog, either by stomach tube or intravenously, there follows a prompt increase in the relative volume of serum. This relative hydremia is roughly pro-

portional to the quantity of glucose introduced. As the relative volume of serum increases there occurs a fall in the chlorine concentration of the serum. The reduction of serum chlorine is too great to be accounted for by a simple dilution of the plasma. If only a moderate quantity of glucose is given, there is practically no change observed in the conductivities of the serum. They remain practically constant in spite of the variations in serum chlorine concentration. This, of course, has produced further examples of the type of "conductivity-chloride discrepancy" mentioned by Atchley, Loeb, Benedict, and Palmer (2). (See last three determinations of the quantities in Fig. 1.) If a large quantity of glucose is administered, there follows a depression of the serum conductivity that is large in proportion to the fall in concentration of serum chlorine, producing the transient type of conductivity-chloride discrepancy identical with those reported previously on human sera (1). In addition to these, a third type of conductivity-chlorine discrepancy has been observed in the serum of one dog. In this variety the serum conductivity is far below the expected, in relation to the existing serum chlorine concentration. It is illustrated by the first two determinations of the quantities in Fig. 2. Inasmuch as it occurred in a normal dog before any non-electrolyte was added to its blood stream, the cause is entirely unknown.

When the administration of glucose is discontinued the relative volume of serum gradually falls to the normal, and the serum chlorine concentration gradually rises again to normal. If the transient type of conductivity-chlorine discrepancy has been present, it disappears. From these observations we find that, so far as blood concentration and water balance are concerned, the experimentally hyperglycemic dog reproduces the conditions known to exist in the elderly or arteriosclerotic diabetic patient.

Studies on Corpuscles.

During the time of intravenous glucose administration there is a transfer of chlorine from the plasma to the corpuscles. Frequently there has been noted a transient decrease in corpuscular chlorine just before the main increase. The significance of this is not clear.

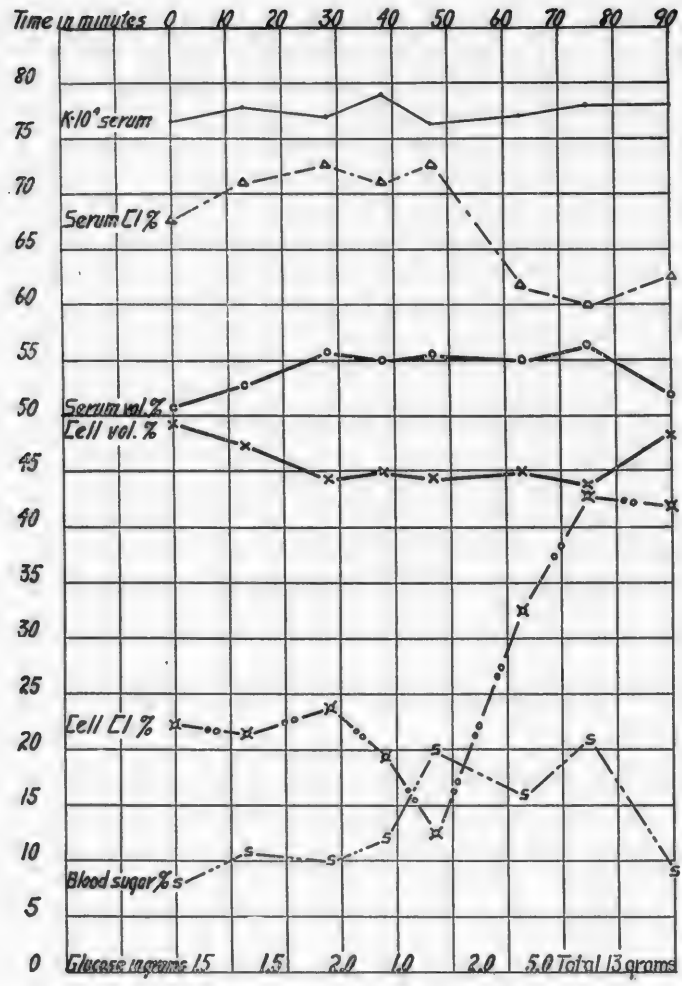

FIG. 1. The effects of a moderate hyperglycemia on the blood of a dog. Note the increase in the relative volume of serum, the reduction of the serum chlorine, and the 100 per cent increase in the corpuscular chlorine. The initial fall in corpuscular chlorine is quite marked. The last three values for serum conductivity and serum chlorine illustrate the first type of conductivity-chloride discrepancy.

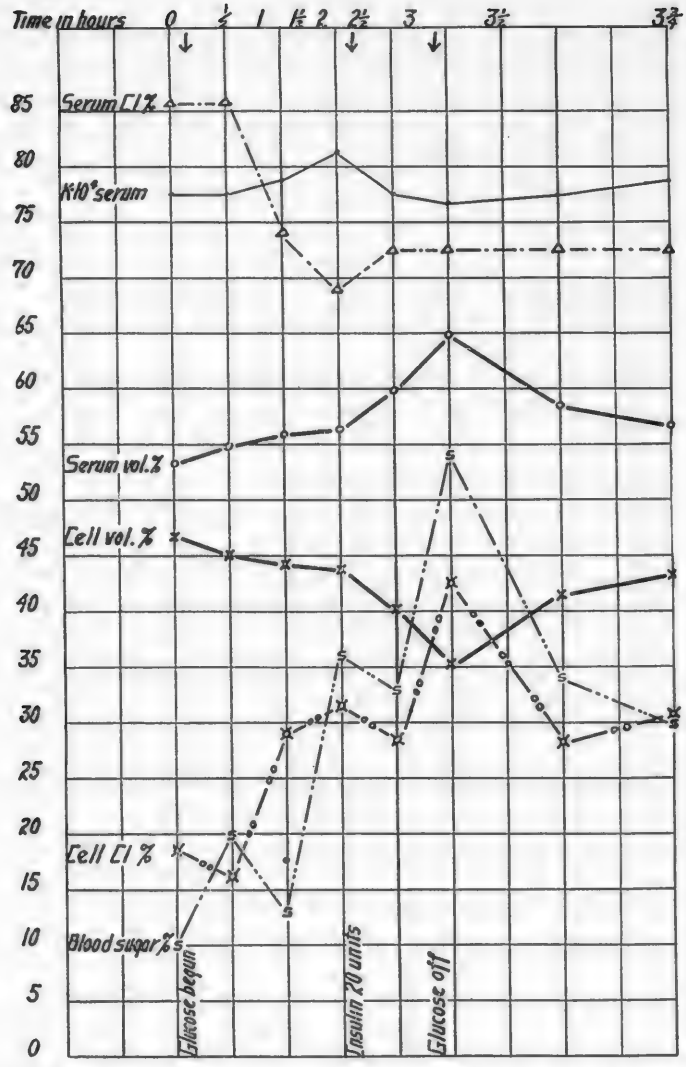

FIG. 2. The effects of a marked hyperglycemia on the blood of a dog. Note the increase in the relative volume of serum, the reduction of the serum chlorine, and the 130 per cent increase in the corpuscular chlorine. The effect of insulin upon the relative blood volumes, blood sugar, and corpuscular chlorine is also shown. The first two values for serum conductivity and serum chlorine illustrate the third type of conductivity-chloride discrepancy.

It is strikingly similar to the transient initial increase in corpuscular chlorine just before the main decrease which was observed in diabetes when the blood sugar was lowered rapidly. Figs. 1 and 2 demonstrate very clearly this phenomenon of chlorine transfer. In general it has been found that the curves for corpuscular chlorine and relative serum volume parallel the blood sugar curve.

The erythrocytes did not increase their average individual volume as a result of the increasing hyperglycemia in the three instances in which the feature was studied. In fact, in Dog 4 the volume diminished as the blood sugar increased despite a 37 per cent reduction in the alkaline reserve, and in Dog 5 the volume increased as the blood sugar fell and then decreased as the blood sugar rose again. In Dog 6 there seemed to be no consistent relation.

The apparatus and methods used in making this series of determinations are identical with those previously used on human blood. The relative volumes of serum and cells were calculated from the electrical conductivities of the whole blood and of blood serum according to the method developed by Stewart (3). Erythrocyte counts were all made by me in duplicate, and with the same pipette and counting chamber. The average erythrocyte volume was obtained by dividing the relative volume of cells, expressed in c. mm., by the number of cells per c. mm. The corpuscular chlorine was calculated from the determined percentage concentrations in the whole blood and in the serum, and from the calculated relative blood volumes. Blood sugar determinations were made by the method of Folin and Wu (4). Blood chloride and serum chloride determinations were made by the method of Whitehorn (5).

The glucose was prepared as a 50 per cent solution in distilled water and then titrated to neutrality with 0.1 normal NaOH, with phenolphthalein as indicator. This precaution is necessary as solutions of glucose in water are quite acid, and their intravenous use may produce results that are not due to glucose alone. Usually it requires about 10 cc. of 0.1 normal NaOH to neutralize 50 gm. in 100 cc. distilled water.

SUMMARY.

1. In normal dogs experimental hyperglycemia causes a prompt dilution of the circulating blood. This is evidenced by the increase in the relative volume of serum and the reduction of the erythrocyte counts. If the hyperglycemia is large, the viscosity of the blood is visibly diminished.

2. As the hyperglycemia increases there occurs a reduction of the serum chlorine concentration and an increase in the concentration of corpuscular chlorine.

3. Reduction of an artificial hyperglycemia restores the relative blood volumes to their normal status. The erythrocyte count rises to the normal and the corpuscular chlorine concentration is diminished. If the reduction is a large one, the viscosity of the blood is visibly increased.

4. The same types of conductivity-chloride discrepancy that occur in human blood are found in the blood of dogs. In addition, a third type is described.

5. Hyperglycemia does not cause the same changes in the volume of the average erythrocyte of the dog as it does in the case of human erythrocytes.

Protocol 1.

June 20, 1924.

Time.	Glucose.	$K \times 10^4$ at 5°C.		Relative volume per cent.		Increase in serum volume per cent.	Blood sugar.	Blood chloride.	Serum chloride.	Corpuscular chloride.
		Blood.	Serum.	Serum.	Cells.					
p.m.	*gm.*						*per cent*	*per cent*	*per cent*	*per cent*
3.40		26.3	76.6	50.68	49.52		0.08	0.452	0.676	0.222
3.44	1.5									
3.53		28.2	77.8	52.82	47.18	2.14	0.11	0.478	0.712	0.216
3.56	1.5									
4.08		29.9	77.1	55.91	44.09	5.23	0.10	0.511	0.726	0.238
4.10	2.0									
4.18		30.3	79.1	55.04	44.96	4.36	0.12	0.480	0.712	0.196
4.20	1.0									
4.27		29.6	76.6	65.75	34.25	15.07	0.20	0.520	0.726	0.126
4.35	2.0									
4.43		29.3	77.2	54.91	45.09	4.23	0.16	0.485	0.618	0.323
4.45	5.0									
4.52							0.23	0.528		
4.55		30.7	78.0	56.36	43.64	5.68	0.21	0.528	0.602	0.432
5.10		27.7	78.0	51.94	48.06	1.26	0.09	0.528	0.627	0.421

Dog 1.—Female; weight 12 kilos. Novocaine anesthesia to expose jugular. Given glucose intermittently in small amounts by intravenous injection. Glucose in 50 per cent solution, made neutral to phenolphthalein by adding sufficient 0.1 N NaOH. Total amount given was 18 gm., representing an approximate rate of 1.1 gm. per kilo body weight per hour.

Protocol 2.

June 25, 1924.

Time.	Glucose.	$K \times 10^4$ at 5°C.		Relative volume per cent.		Increase in serum volume per cent.	Blood sugar.	Blood chloride.	Serum chloride.	Corpuscular chloride.
		Blood.	Serum.	Serum.	Cells.					
a.m.	*gm.*						*per cent*	*per cent*	*per cent*	*per cent*
11.33		29.7	78.0	54.84	45.16		0.09	0.490	0.680	0.259
11.47	5.0									
11.55		32.2	78.8	57.87	42.13	3.03	0.14	0.490	0.680	0.229
m.										
12.00	5.0									
p.m.										
12.08		33.5	78.1	60.15	39.85	5.81	0.16	0.530	0.680	0.304
12.13	5.0									
12.16		34.9	77.9	62.31	37.69	7.47	0.22	0.530	0.690	0.268
3.10		31.2	79.4	56.0	44.0	1.16	0.09	0.503	0.700	0.252
3.15	5.0									
3.25		34.2	77.2	61.80	38.20	6.96	0.14	0.520	0.690	0.245
3.30	5.0									
3.35		35.9	78.8	62.86	37.14	8.02	0.17	0.528	0.700	0.237
3.42	5.0									
4.21		33.4	79.4	59.06	40.94	4.22	0.10	0.510	0.660	0.294
4.25	5.0									
5.15		32.9	80.5	57.57	42.43	2.73	0.09	0.580	0.660	0.471

Dog 2.—Female; weight 13 kilos. Novocaine anesthesia to expose jugular. Intermittent intravenous injections of equal amounts of glucose (50 per cent neutral solution). Total amount given was 35 gm., representing an approximate rate of 1.55 gm. per kilo body weight per hour.

Protocol 3.

July 11, 1924.

Time.	Glucose.	$K \times 10^4$ at 5°C.		Relative volume per cent.		Increase in serum volume per cent.	Blood sugar.	Blood chloride.	Serum chloride.	Corpuscular chloride.	Rectal temperature.
		Blood.	Serum.	Serum.	Cells.						
a.m.							*per cent*	*per cent*	*per cent*	*per cent*	°C.
11.35		28.5	77.4	53.53	46.47		0.10	0.544	0.858	0.182	38.3
11.45	Begun.										
p.m.											
12.06	(15.5 gm.)	29.5	77.7	54.82	45.18	1.29	0.20	0.544	0.858	0.163	
12.50	(37.0 ")	30.8	78.9	55.98	44.02	2.45	0.18	0.544	0.742	0.292	38.3
1.50	(59.0 ")	32.3	81.6	56.13	43.87	2.60	0.36	0.528	0.692	0.318	
2.20	(84.0 ")	32.0	77.4	58.78	41.22	5.25	0.33	0.544	0.726	0.285	
2.26	Insulin 20 units.										38.3
2.45	(109 gm.)										
2.51	Stopped (140.0 gm.)										
3.09		36.2	76.6	65.11	34.89	11.58	0.54	0.561	0.726	0.425	
3.10		31.9	77.5	58.55	41.45	5.02	0.34	0.544	0.726	0.287	
3.20		32.6	81.3	56.70	43.30	3.17	0.30	0.544	0.726	0.306	38.3

Dog 3.—Female; weight 23 kilos. Morphine narcosis. Continuous injection of 50 per cent neutral glucose by means of a Woodyatt apparatus. Total amount injected was 140 gm. Injection time 3 hours and 45 minutes. Glucose injected at a rate of 2.3 gm. per kilo body weight per hour. Total fluid added to the circulating blood was 280 cc.

Protocol 4.

Dec. 29, 1924.

Time.	Glucose.	$K \times 10^4$ at 5°C.		Relative volume per cent.		Increase in serum volume per cent.	R.B.C. per c.mm.	Average R.B.C. volume.	Blood sugar.	Blood chloride.	Serum chloride.	Corpuscular chloride.	Plasma CO_2.
		Blood.	Serum.	Serum.	Cells.								
p.m							*millions*	*c. μ*	*per cent*	*per cent*	*per cent*	*per cent*	*vol. per cent*
2.00		34.8	80.0	60.56	39.44		6.685	59.0	0.13	0.511	0.643	0.308	62.0
2.15	Begun.												
2.30	(50.0 gm.)	44.9	74.2	78.20	21.80	17.64	4.920	44.3	0.60	0.511	0.594	0.213	58.0
2.45	(80.0 ")	44.3	69.9	82.0	18.0	21.44	5.250	34.3	0.60	0.478	0.594	?	48.0
2.52	Insulin 20 units.												
3.20	Stopped (97 gm.)												
3.25		45.6	70.6	83.0	17.0	22.44	4.750	35.8	0.75	0.529	0.643	?	39.0

Dog 4.—Male; weight 9.5 kilos. Novocaine anesthesia to expose jugular. Continuous injection of 50 per cent neutral solution of glucose. Total amount injected was 97 gm., over an injection time of 70 minutes, representing a rate of 10.4 gm. per kilo body weight per hour. Diuresis of 650 cc. at 2.55 p.m. Urine contained 6.2 per cent glucose. Total fluid added to the circulating blood was 194 cc. Exitus occurred during the evening of the same day.

Protocol 5.

Feb. 23, 1925.

Time.	Glucose.	$K \times 10^4$ at 5°C.		Relative volume per cent.		Change in serum volume per cent.	R.B.C. per c. mm.	Average R.B.C. volume.	Blood sugar.	Blood chloride.	Serum chloride.	Corpuscular chloride.
		Blood.	Serum.	Serum.	Cells.							
a.m.							*millions*	*c. μ*	*per cent*	*per cent*	*per cent*	*per cent*
11.45	25.0 gm.											
m.												
12.00		31.9	72.7	62.38	37.62		7.085	53.1	0.50	0.528	0.676	0.282
p.m.												
12.10	Insulin 50 units.											
12.22		31.4	78.4	57.0	43.0	−5.38	7.780	55.2	0.30	0.494	0.749	0.156
12.37		31.1	80.2	55.5	44.5	−6.9	8.090	55.0	0.21	0.528	0.764	0.234
1.05	25.0 gm.											
1.08		30.5	79.4	54.96	45.04	−7.4	8.020	56.1	0.136	0.560	0.745	0.334
1.20		31.8	72.3	62.57	37.43	+0.2	6.840	54.7	0.375	0.594	0.775	0.148

Dog 5.—Female; weight 12 kilos. Novocaine anesthesia to expose jugular. Given 50 cc. of a neutral 50 per cent solution of glucose intravenously before the first blood sample was taken. Then given 50 units of insulin intramuscularly. A severe insulin reaction began at 12.30 p.m., reached the stage of clonic muscular spasms at 1.00 p.m., and was relieved by the intravenous injection of 25 gm. glucose and 1 cc. adrenalin subcutaneously.

Protocol 6.

Feb. 27, 1925.

Time.	Glucose.	$K \times 10^4$ at 5°C.		Relative volume per cent.		Change in serum volume per cent.	R.B.C. per c. mm.	Average R.B.C. volume.	Blood sugar.	Blood chloride.	Serum chloride.	Corpuscular chloride.
		Blood.	Serum.	Serum.	Cells.							
p.m.							*millions*	*c.μ*	*per cent*	*per cent*	*per cent*	*per cent*
12.15		32.9	79.4	58.6	41.4		7.450	55.5	0.12	0.594	0.693	0.454
12.40	25.0 gm.											
12.52	Insulin 25 units.											
12.54		38.6	74.6	70.0	30.0	+11.4	5.610	53.5	0.40	0.544	0.726	1.200
1.13		34.6	77.5	62.2	37.8	+3.6	6.340	59.6	0.30	0.610	0.745	0.388
1.19		34.1	79.4	60.2	39.8	+1.6	7.070	56.3	0.20	0.577	0.774	0.280
1.28		32.9	78.7	58.8	41.2	+0.2	8.070	51.1	0.30	0.594	0.774	0.337

Dog 6.—Female; weight 12.5 kilos. Novocaine anesthesia to expose jugular. Given 50 cc. of a 50 per cent solution of glucose neutral to phenolphthalein. Then given 25 units of insulin. A very mild insulin reaction began at 1.10 p.m., consisting of salivation, restlessness, muscular twitchings, and irregular respirations. There were no muscular spasms or convulsions. The dog made a prompt recovery without glucose or other aid.

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STUDIES ON UROBILIN PHYSIOLOGY AND PATHOLOGY.

IV. Urobilin and the Damaged Liver.

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(Received for publication, May 7, 1925.)

Following the discovery of urobilin in 1868, recognition of it in the urine of patients aroused great interest. Urobilinuria was observed in a wide variety of clinical conditions,[1] and many studies were reported of its pathological significance and diagnostic value. It was found present in noteworthy quantity in two general types of disease: those characterized by an increased destruction of red cells, and those involving actual damage to the liver parenchyma or occlusion of the bile ducts. Of great importance for the clinical interpretation of such a finding is the determination of what is responsible therefor within the organism. Despite the variety of hypotheses advanced concerning the physiology of urobilin in the normal and diseased animal, [1-4] relatively little that is positive has been ascertained.

Earlier papers of this series have dealt with the origin and fate of urobilin under more or less normal conditions.[5-7] The present communication will be concerned with the happenings when there has been direct injury to the hepatic cells or obstruction to the ducts.

We have shown that, under normal conditions, urobilin is formed in the dog only within the intestinal tract. Drainage of the entire bile quantity under sterile conditions, or retention of it by common duct occlusion results in the

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prompt disappearance of the pigment from the excreta, and from the bile. The animal becomes in fact urobilin-free. But whenever bilirubin enters the intestine, urobilin is rapidly formed therefrom, part of which escapes in the stool, while another part is absorbed from the gut, either as such or in the form of related pigments,[7] and makes its appearance in the bile. This secondary resorption and resecretion play an important part in the normal fate of the pigment, and, as will now be shown, in the findings when the liver has been injured.

Methods.

The present experiments were performed on the same series of dogs used in studies already reported.[5–7] In some of the animals the common duct had been intubated according to the method of Rous and McMaster;[8] in others a branch of the hepatic duct only was cannulated, but according to the same method, and the bile drained in this way from one or two liver lobes, whilst the remainder, from about two-thirds of the liver as a rule, reached the duodenum along the normal path. The preparation of the animals as well as the methods used for the daily collection and pigment estimation on urine, stool, and bile have been previously described.[5–7] The influences of two general types of hepatic disorder were studied: the one brought about by the administration of toxic substances, the other by the occlusion of bile ducts. In judging the degree of liver injury in the animals which survived the principal criteria have been the nature and extent of the biliary disturbances as disclosed by the jaundice, or by changes in the bile and urine collected, or by both.

Hepatic injury was produced by the administration of chloroform, phosphorus, amyl alcohol, and toluylenediamine. In general the amounts given were not large enough to cause a serious general intoxication. Aside from marked jaundice, there was, in many instances, no striking bodily disturbance. In the case of amyl alcohol, however, even moderate doses (2 cc. per kilo given with ethyl alcohol by gavage) resulted in a marked toxemia in the absence of jaundice. Use of it was discontinued for this reason. Yellow phosphorus was given by stomach tube in doses averaging 13 mg. per kilo dissolved in olive oil. Toluylenediamine was given to the amount of 12 mg. per kilo, in aqueous solution, by gavage. It caused hemolysis and frequently a marked depression in the bile output and alterations in its constituents, 1 to 3 days after administration.

That chloroform has a specific damaging effect on the hepatic parenchyma is

[8] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1923, xxxvii, 11.

well known.[9,10] The dietary factor in the injury is an important one.[11] In our animals, on uniform standard diets, the anesthetic was given always at the same time, 5 hours after feeding. As a rule, 75 cc. given by inhalation over a period of 45 minutes to a 10 kilo dog, causes marked changes in the bile, changes already described in a paper from this laboratory by Drury and Rous.[12] The secretion is lessened in amount and in content of biliary constituents to such an extent, sometimes, that the fluid elaborated is a "white bile." The general condition of the animal is often good, despite the disturbance, but the margin of safety in such instances is narrow, at least in intubated animals losing all of the bile. In some animals the respirations would cease permanently at almost the first whiff of chloroform. It was found best, therefore, to bring the animal under anesthesia with ether, changing immediately thereafter to chloroform.

When liver disturbance was to be produced by biliary obstruction, the ducts were severed between ligatures under ether anesthesia, or the outlet tube of intubated animals was clamped. Not a few cases of biliary obstruction were studied in which, some time after intubation, the cannula had become plugged with mucus or stone, or the collecting tube had become bent sharply upon itself, thus effecting either a partial or a total obstruction, according as the tube was draining the entire liver or only a portion of it.

The findings after direct hepatic injury with the toxic substances just discussed, and those after biliary obstruction, will be examined separately. The data have been further subdivided into those obtained from dogs draining all of the bile, and those losing but a fraction thereof, since in the one case the body fluids and excreta are urobilin-free, while in the other bile and stool both contain the pigment, just as do intact animals.

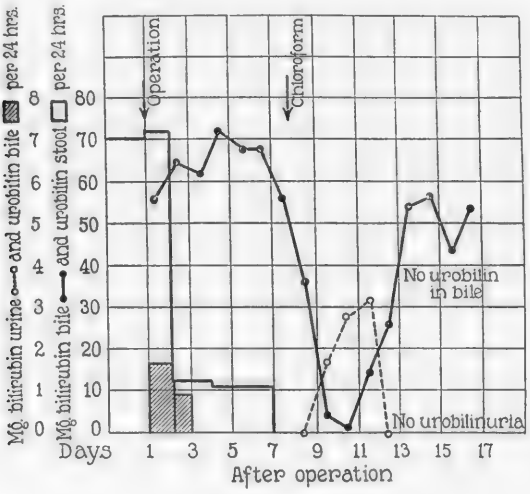
Only animals yielding sterile bile were used in these experiments. The daily bile specimens were tested regularly for infection, every day or two, by incubating portions on agar. In addition, the stained sediment from centrifuged specimens was occasionally examined. When bacteria were found the dog was discarded for the purposes of this work.

Absence of Urobilinuria during Liver Injury under Conditions of Total Bile Loss.

The complete disappearance of urobilin from the bile and feces, 2 to 4 days after intubation of the common duct for bile collection,

[9] Whipple, G. H., and Sperry, J. A., *Bull. Johns Hopkins Hosp.*, 1909, xx, 278.
[10] Howland, J., and Richards, A. N., *J. Exp. Med.*, 1909, xi, 344.
[11] Davis, N. C., and Whipple, G. H., *Arch. Int. Med.*, 1919, xxiii, 612.
[12] Drury, D., and Rous, P., *J. Exp. Med.*, 1925, xli, 611.

has been reported in a previous paper.[5] Under these circumstances of continued bile loss the bile and feces were found to remain urobilin-free for as long as $4\frac{1}{2}$ months, the longest period of observation. Ten instances have now been studied of hepatic injury in animals rendered urobilin-free in this way. The results as regards urobilin were uniform in all, regardless of the injurious agent employed, or



TEXT-FIG. 1. Effects of liver injury by chloroform in a dog losing the total bile.

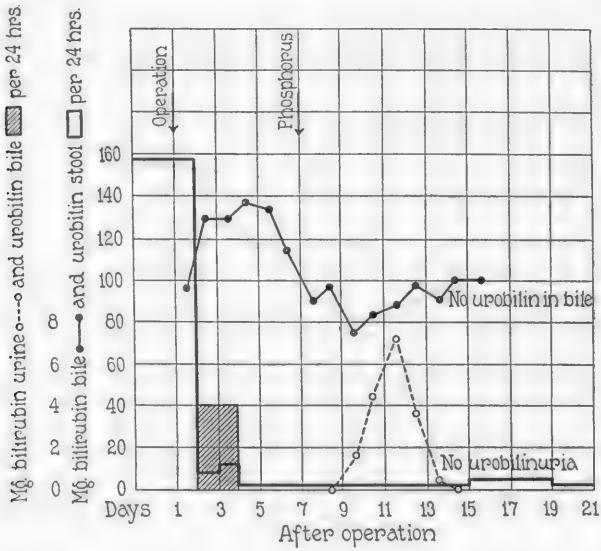
The severity of the hepatic damage is indicated by the drop in the amount and bilirubin content of the bile, which, on the 3rd day after anesthesia, had become almost colorless. At the time of greatest disturbance there was a moderate bilirubinuria. No urobilin appeared in the bile or stool (see Protocol I).

the degree of liver damage it induced. In no instance did the pigment reappear in bile or stool despite the frequently intense icterus; and there was never any urobilinuria. Rarely, small amounts of the pigment appeared in the stool, a finding which is to be accounted for by the excretion from the jaundiced body fluids of small amounts of bilirubin into the large bowel.[5]

Specimen Protocols.

I. Female dog, weight 9 3/4 kilos (see Text-fig. 1).

8 days prior to the experiment the common duct was intubated under ether. Recovery was uneventful, and the stools and bile became urobilin-free on the 2nd day. Chloroform was then given by inhalation for 50 minutes, 40 cc. being used. The 24 hour output of bilirubin for the period immediately preceding the anesthesia was 68 mg. The specimen of the next 24 hours contained only 34 mg.; and during


Mg. bilirubin urine o---o and urobilin bile ▨ per 24 hrs.
Mg. bilirubin bile ●—● and urobilin stool □ per 24 hrs.
Operation
Phosphorus
No urobilin in bile
No urobilinuria
Days
After operation

TEXT-FIG. 2. Effects of liver injury with phosphorus in a dog losing the total bile.

The hepatic disturbance, as indicated by the changes in the bile, was moderate, but bilirubinuria and tissue icterus developed. No urobilin was found at any time (see Protocol II).

the following 24 hours the liver excreted but 6.6 mg. On the succeeding day the collecting balloon held only 12 1/2 cc. of "white bile," and there was definite tissue icterus with moderate bilirubinuria. The stools remained clay-colored throughout the observations.

Now bile secretion was rapidly resumed. Within another week icterus had disappeared, the urine had become free of bilirubin, and the bile had the ordinary

character of the secretion elaborated some days after intubation. It was never again so rich in bilirubin as shortly after operation, for reasons that have been given elsewhere.[13,14]

The urine at no time showed the slightest trace of urobilin, the stool remained free from it, and none was present in the bile even at the height of the liver injury.

II. Male dog, weight 15 1/4 kilos (see Text-fig. 2).

Phosphorus was given, 200 mg. in olive oil, by stomach tube, 6 days after intubation, under ether, of the common duct. The animal had become urobilin-free on the 4th day after operation, and remained so. The bilirubin content of the bile prior to administration of the phosphorus was approximately the same from day to day, about 110 mg., during each 24 hours. Thereafter no serious symptoms developed, and the biliary disturbance was only moderate, reaching its height in 3 days, when the 24 hour bile specimen contained but 76 mg. of bilirubin. There were scleral icterus and a mild bilirubinuria. The return to "normal" was complete in 9 days.

Throughout the period of the hepatic disturbance the bile and urine remained free of urobilin. The stool continued to show a trace of the pigment, never exceeding 4 mg. per day.

Urobilinuria Following Liver Injury under Conditions of Partial Bile Loss.

Eight instances of hepatic damage were studied in animals losing but a fraction of the total bile as the result of the intubation of a small hepatic duct. The effects of the injury on the bile could be followed directly in the daily sample obtained. Under the experimental conditions, with most of the bile still flowing to the intestine, urobilin was a regular constituent both of bile and stool. The liver injury was induced by chloroform, phosphorus, or toluylenediamine. Urobilinuria followed in every case, its duration and degree depending on the severity of the pathological change.

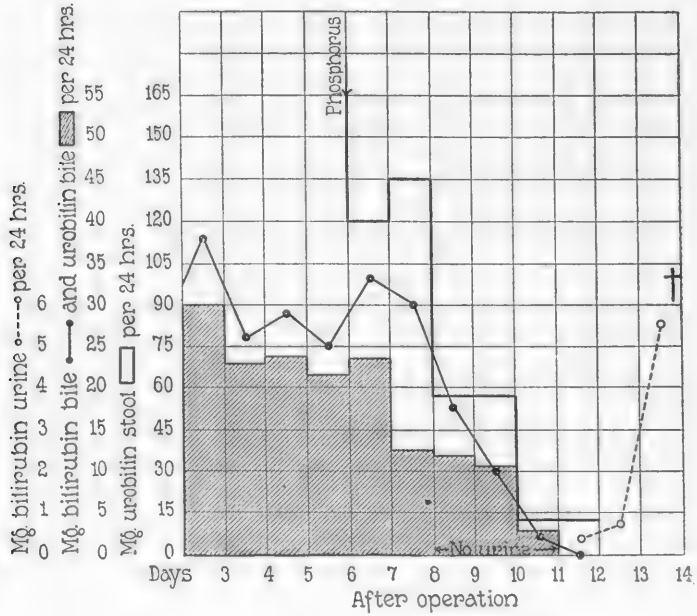
Specimen Protocols.

III. Male dog, weight 12 1/4 kilos (see Text-fig. 3).

Under ether anesthesia the duct to the left lateral lobe was intubated, and 31 per cent of the liver drained, as the eventual necropsy showed. 6 days after the operation the bile had come to contain approximately constant amounts of urobilin and bilirubin from day to day, and the urine was urobilin-free. At this

[13] McMaster, P. D., Broun, G. O., and Rous, P., *J. Exp. Med.*, 1923, xxxvii, 685.
[14] Broun, G. O., McMaster, P. D., and Rous, P., *J. Exp. Med.*, 1923, xxxvii, 733.

time 200 mg. of phosphorus, dissolved in olive oil, was given by stomach tube. 3 days later the urobilin in the fraction of the bile studied had fallen to half its previous amount, and the output of bilirubin from 30 to 13 mg. in 24 hours.


Mg. bilirubin urine ----- per 24 hrs.
Mg. bilirubin bile —— and urobilin bile ▨ per 24 hrs.
Mg. urobilin stool □ per 24 hrs.
Phosphorus
No urine
Days
After operation

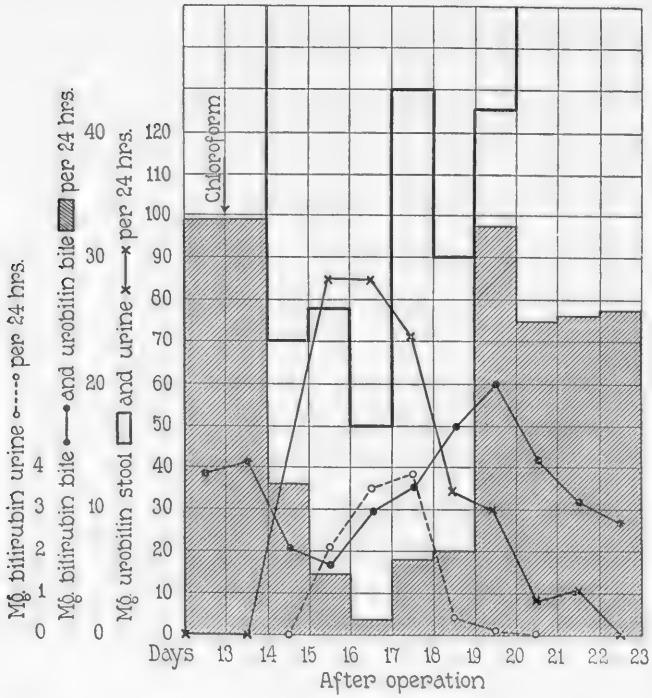
TEXT-FIG. 3. Effects of fatal hepatic injury with phosphorus in a dog losing but a fraction of the total bile.

The urine was urobilin-free prior to the giving of phosphorus, but soon afterwards showed the pigment in increasing amounts. There were several days during which no urine was voided spontaneously. Urobilin quantitation was not attempted during this period, but tests were made on small, catheterized specimens obtained from time to time. Spontaneous voiding was resumed on the 12th day, by which time the liver was secreting only "white bile," devoid of bilirubin. The urine now became urobilin-free, just as when all bile is diverted from the intestine. With the bile suppression bilirubinuria developed and became intense (see Protocol III).

Catheterized urine specimens, procured on each of the succeeding 3 days, revealed the existence of an intense urobilinuria, whereas previously the pigment had been absent.

The biliary disturbance rapidly grew marked, eventuating in bile suppression;

the stools became acholic, and intense tissue icterus developed. Now the urine voided contained no urobilin, only bilirubin, and the disappearance of the urobilin


Mg. bilirubin urine ----- per 24 hrs.
Mg. bilirubin bile —— and urobilin bile ▨ per 24 hrs.
Mg. urobilin stool □ and urine ——× per 24 hrs.
Chloroform
Days
After operation

TEXT-FIG. 4. Effects of moderate liver injury from chloroform in a dog losing but a fraction of the total bile.

Evidence of the hepatic damage is to be found in the drop in the bilirubin content of the bile to half the ordinary amount. Since the damage never resulted in the development of "white bile," one may assume that the pigment was at all times reaching the duodenum (see Protocol IV). Urobilinuria developed promptly and persisted for several days. It may be recalled (Text-fig. 3, as also Text-fig. 5) that when there is complete bile suppression the urine, containing urobilin so long as bile continues to be secreted after liver damage develops, becomes urobilin-free.

was practically synchronous with the development of the bile suppression. The animal died next day, the 8th after the phosphorus had been given. Autopsy revealed a typical phosphorus liver, large, pale, and fatty. The bile ducts were

practically empty, only the finer radicals containing a little dark, viscid bile. No signs of infection were anywhere visible, and culture from the liver on agar and in broth proved sterile.

IV. Male dog, weight 10 3/4 kilos (see Text-fig. 4).

Intubation of the duct from the left lateral lobe was performed under ether anesthesia, and thus we were enabled to collect daily the bile from 27 per cent of the liver. The secretion contained a uniform amount of urobilin and bilirubin from day to day. After 2 weeks, 60 cc. of chloroform was given by inhalation, the administration of the anesthetic lasting 50 minutes. 3 days later the 24 hour output of bilirubin in the bile sample had dropped from 11 to 4 mg., and biliary urobilin had likewise greatly diminished. Recovery, with reestablishment of a more normal biliary secretion, now rapidly took place, being to all appearances complete within another week.

The urine, previously urobilin-free, showed this pigment the day after the administration of chloroform, and there was pronounced urobilinuria for 5 days more, disappearing only on the 8th day. Bilirubinuria on the contrary was moderate and lasted but 4 days.

V. Male dog, weight 10 3/4 kilos (see Text-fig. 5).

30 days after the intubation, under ether, of the duct from the left lateral lobe, draining 27 per cent of the liver, toluylenediamine was given by gavage, 120 mg. in aqueous solution. 2 days later the bilirubin content of the bile had dropped to one-fifth of the previous quantity and, in the succeeding 24 hours, only 4 cc. of "white bile" assembled in the collecting balloon. Resumption of bile secretion soon followed.

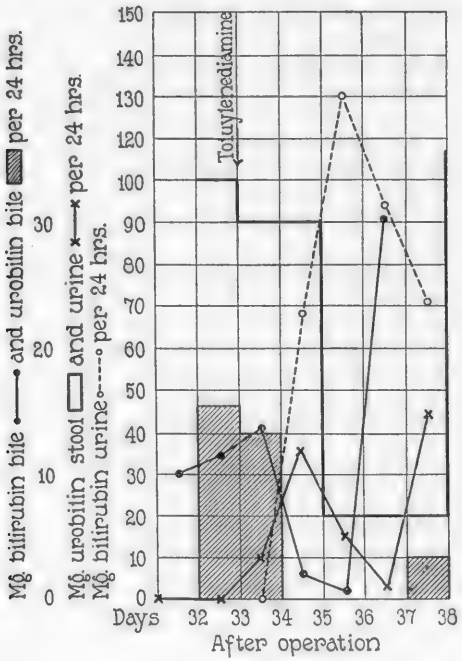
Marked urobilinuria was found the day after the toluylenediamine had been given, and it continued for 2 more days. With the suppression of secretion, as indicated by the finding of "white bile," and the consequent failure of bile pigment to reach the intestine, it diminished and disappeared, only to reappear again upon resumption of the bile flow.

Absence of Urobilinuria during Total Biliary Obstruction.

When all of the bile is diverted from the intestine, and lost to the body, as after intubation of the common duct, urobilin disappears from bile, stool, and urine.[5] But what will happen when the bile constituents are prevented from reaching the intestine and accumulate within the body, as happens during total biliary obstruction? Under such circumstances there is not only a general disturbance of the organism, but a progressively increasing injury to the liver, with secondary cirrhosis.[15] Nevertheless, one finds but a transient initial

[15] Rous, R., and Larimore, L. D., *J. Exp. Med.*, 1920, xxxi, 609; xxxii, 249.

urobilinuria referable to the presence of the pigment in the intestine at the time when the obstruction is produced by operation. Thereafter the urine is free from the pigment, while the slight traces of it appearing in the stool have an obvious source in the bilirubin passing



TEXT-FIG. 5. Effects of severe liver injury from toluylenediamine in a dog losing only a fraction of the total bile.

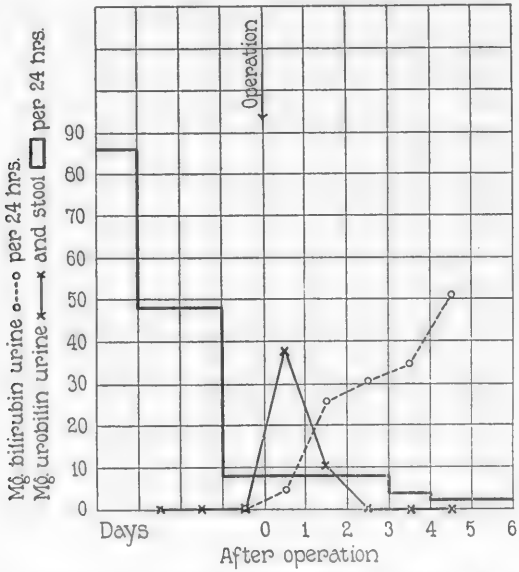
Practically complete biliary suppression developed in this instance. The initial urobilinuria, synchronous in appearance with the beginning of the liver derangement, disappeared when bile no longer reached the intestine, only to reappear when bile flow was resumed (see Protocol V).

into the gut through the jaundiced mucosa.[5] Where obstruction is induced in an animal previously rendered urobilin-free by intubation of the common duct, the pigment fails to appear at all, despite the development of marked bilirubinuria.

Specimen Protocols.

VI. Male dog, weight 10 3/4 kilos (see Text-fig. 6).

Under ether anesthesia, the common duct was cut between ligatures, the cystic duct treated similarly, and the gall bladder evacuated. On the next day the urine contained bilirubin, and 3 days later tissue icterus developed, the stool becoming acholic at the same time, but containing traces of urobilin not exceeding 4 mg. per 24 hours.



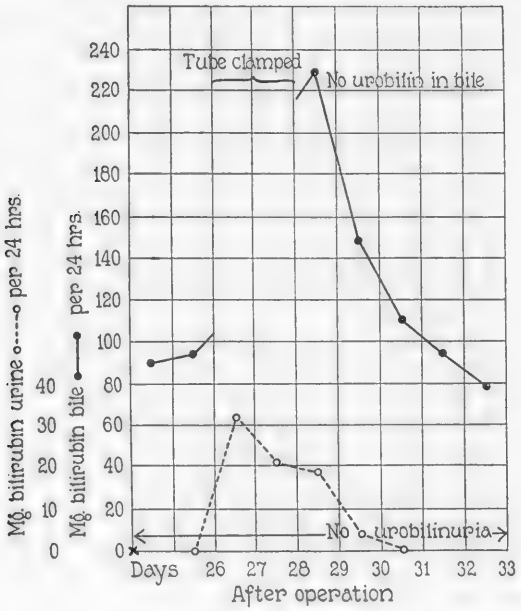
TEXT-FIG. 6. Transient urobilinuria following the operation to produce total biliary obstruction.

The disappearance of the urobilinuria was prompt, and after the 3rd day none was found despite the persisting obstruction, intense jaundice, and marked bilirubinuria (see Protocol VI).

The urine was free from urobilin prior to the operation, but in the succeeding 24 hour period it contained 40 mg., during the second 24 hours, 11 mg., and none at any time thereafter. On the 6th day after operation the animal was noted to have developed a respiratory infection, and it was killed with chloroform. The usual picture of biliary obstruction was found: an enlarged, jaundiced liver and dilated, tortuous ducts filled with "white bile." Cultures from this fluid, as well as from the liver itself, in broth and agar, proved sterile.

VII. Male dog, weight 18 kilos (see Text-fig. 7).

The common duct had been intubated, under ether, 1 month prior to the experiment. The bile collected daily was sterile, showed no urobilin, and contained, on the average, 85 mg. of bilirubin in each 24 hour specimen. Urine and feces were urobilin-free.



TEXT-FIG. 7. Total biliary obstruction by occlusion of the outlet tube after common duct intubation.

Moderate bilirubinuria developed during the 2 day period of obstruction but no urobilinuria. The stools remained acholic and urobilin-free. Following the relief of the obstruction the bilirubin output of the bile was at first very large, but the secretion contained no urobilin (see Protocol VII).

On the 26th day of intubation total obstruction was produced by clamping the outlet tube for a period of 48 hours. Bilirubinuria developed within 24 hours after the onset of the obstruction, disappearing some 72 hours after it had been released. Urobilinuria failed to develop at any time, and the stools remained pigment-free. The bile, on resumption of flow, contained large amounts of bilirubin, 230 mg. during 24 hours, but the amount gradually fell off to the pre-obstructive level. Urobilin did not appear in it.

Development of Urobilinuria Following Local Biliary Obstruction.

The effects of obstructing bile flow from a portion of the liver only were next studied. Under such circumstances much bilirubin continues to reach the intestine and urobilin to be formed from it. The question whether the local obstruction will result in urobilinuria is an important one. We know that bilirubinuria fails to develop until the ducts from more than three-fourths of the parenchyma have been occluded.[16] The present observations show that urobilin appears in the urine when obstruction of far less degree has been produced.

The obstruction was effected by clamping the outlet tube in dogs draining but a sample of the liver secretion. There were some instances as well in which stone formation or a twist in the tubing dammed back the bile. Ordinarily the obstruction involved about 30 per cent of the hepatic tissue. The finding in all cases was a moderate urobilinuria, without ever any bilirubinuria. The discrepancy cannot be laid to the existence of a higher kidney threshold for bilirubin than for urobilin, since in the dog there is practically no threshold for the first mentioned pigment.[16] It can only be due to a failure of the unobstructed portion of the hepatic tissue to remove urobilin from the blood as completey as it does bilirubin.

Specimen Protocols.

VIII. Male dog, weight 11 kilos (see Text-fig. 8).

3 weeks prior to the obstruction, a duct had been intubated, under ether, draining 31 per cent of the liver, as autopsy later showed. The dog remained in excellent condition and continued to have urobilin in bile and stool.

The outlet tube was clamped during 48 hours, and then released. The urine previously free of urobilin was found to contain 10 mg. during this period, but never showed bilirubin.

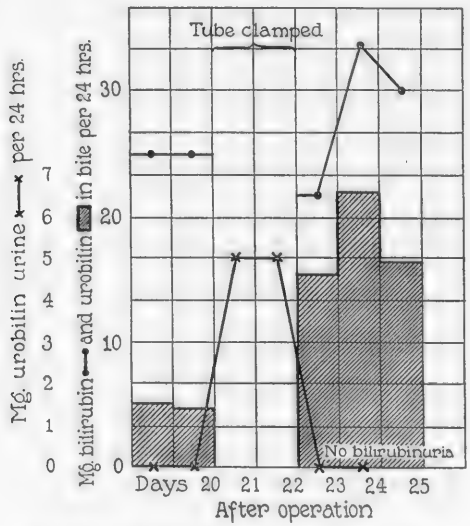
IX. Male dog, weight 19 1/2 kilos (see Text-fig. 9).

An instance of exceptional interest will be recorded, in which the obstruction occurred shortly after a hemoglobin injection.

Under ether, the duct from the left lateral liver lobe was cannulated, and the bile from this approximate third of the hepatic tissue collected, while the secretion from elsewhere flowed uninterruptedly to the intestine. Urobilin continued present in the bile and stool. On the 6th day of intubation 150 cc. of blood was

[16] McMaster, P. D., and Rous, P., *J. Exp. Med.*, 1921, xxxiii, 731.

removed from a vein, laked with 200 cc. of distilled water, and the whole reinjected intravenously. There followed the expected tremendous increase in the bilirubin output. Bilirubinuria and hemoglobinuria were present for 2 days. For the sake of simplicity, these occurrences are not represented on the chart. After 3 further days, the bile from the intubated third of the liver was noted to contain very little bilirubin and urobilin, and to be much diminished in amount, indicating that obstruction was taking place. This obstruction was not a late result of the blood injection, or one involving the whole liver, but local and due to other causes,



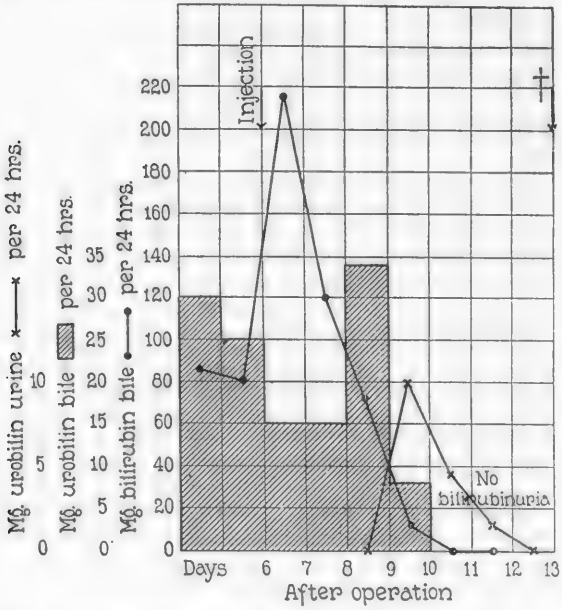
TEXT-FIG. 8. Urobilinuria following obstruction of about one-third of the liver.

The outlet tube draining the liver third was clamped for 2 days. Moderate urobilinuria resulted but no bilirubinuria (see Protocol VIII).

as the autopsy showed. In 3 more days it was complete, only 4 cc. of "white bile" being collected in 24 hours. There was no jaundice.

The chart depicts the changes in urobilin. Urobilinuria, absent during the initial period of derangement after the intravenous injection of hemoglobin, came on as the obstruction developed, only to disappear later. Bilirubinuria was entirely absent. The animal was killed to determine the cause of the obstruction, and the cannula found filled with deeply pigmented stones. The ducts drained by the cannula were dilated and held glairy "white bile." Cultures from this latter proved sterile.

The fact is well attested[15,16] that after obstruction of a duct from a portion of the liver the remaining parenchyma rapidly takes over the function of the occluded portion, and this latter gradually atrophies and disappears. The disappearance of the urobilinuria, despite the persisting obstruction, in the present instance, may, with good reason, be laid to a functional accommodation.

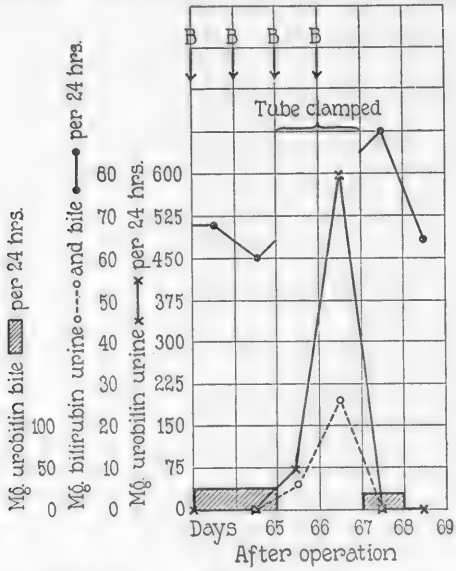


TEXT-FIG. 9. Urobilinuria following obstruction of one-third of the liver. 3 days prior to the development of the obstruction hemolyzed blood had been injected into a vein. No urobilinuria followed, yet it promptly appeared when the cannula draining the duct from a portion of the liver became plugged with stones. No bilirubinuria occurred at any time during the obstruction. Bilirubinuria and hemoglobinuria were found on the 7th and 8th days immediately after the blood injection (see Protocol IX).

Bile Feedings Induce Urobilinuria When the Liver Has Been Damaged.

From the foregoing observations, it is evident that urobilinuria does not occur after liver injury when all bile pigment is excluded

from the intestine, as, for example, when the common duct is drained or obstructed. But what will happen when the animals are fed bile by gavage? The test has been made on a human being by Müller,[17] who found that he could precipitate urobilinuria in a jaundiced



TEXT-FIG. 10. Development of urobilinuria after bile feedings under conditions of total biliary obstruction.

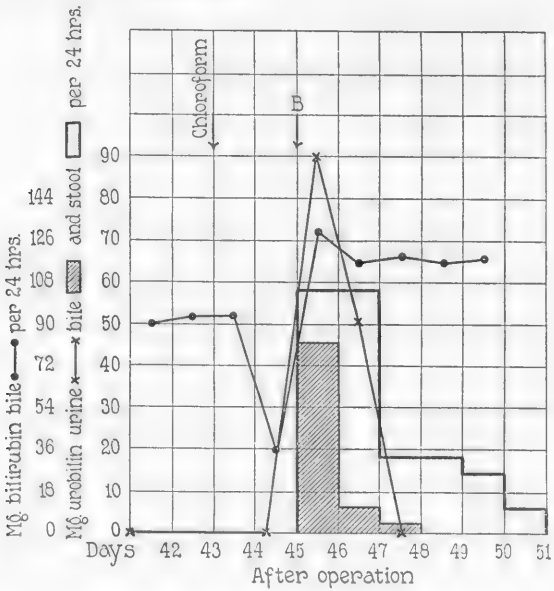
The animal had an intubated common duct. The feedings (B) were begun before biliary obstruction was produced. They brought about the appearance of urobilin in the bile and stool, but not in the urine until after biliary obstruction had been effected by clamping the outlet tube from the liver. The pronounced urobilinuria (600 mg.) which then developed disappeared almost at once with discontinuance of the feedings and relief of the obstruction. Bilirubinuria was mild at most. Compare with Text-fig. 6 (Protocol VI),—the same experiment minus the bile feedings,—in which only bilirubinuria developed (see Protocol X).

patient with common duct obstruction merely by feeding urobilin-free pig bile by mouth.

Ordinarily, the return to the animal of bile lost through an intubated

[17] Müller, Fr., *Verhandl. Cong. inn. Med.*, 1892, xi, 118.

common duct brings about the reappearance of urobilin in the bile and feces, as reported in a preceding paper.[6] But in the absence of hepatic damage urobilinuria is not induced. In the experiments now to be described, in which the liver had been injured, urobilinuria regularly followed the feedings.



TEXT-FIG. 11. Urobilinuria following a bile feeding to a dog losing the total bile and with an injured liver.

The injury by chloroform did not of itself cause urobilin to appear in bile or urine (see also Text-fig. 1), but large amounts of the pigment appeared temporarily in both secretions after bile had been administered by gavage (B). The gavage was done at a time when the liver injury was at its height[9] (see Protocol XI).

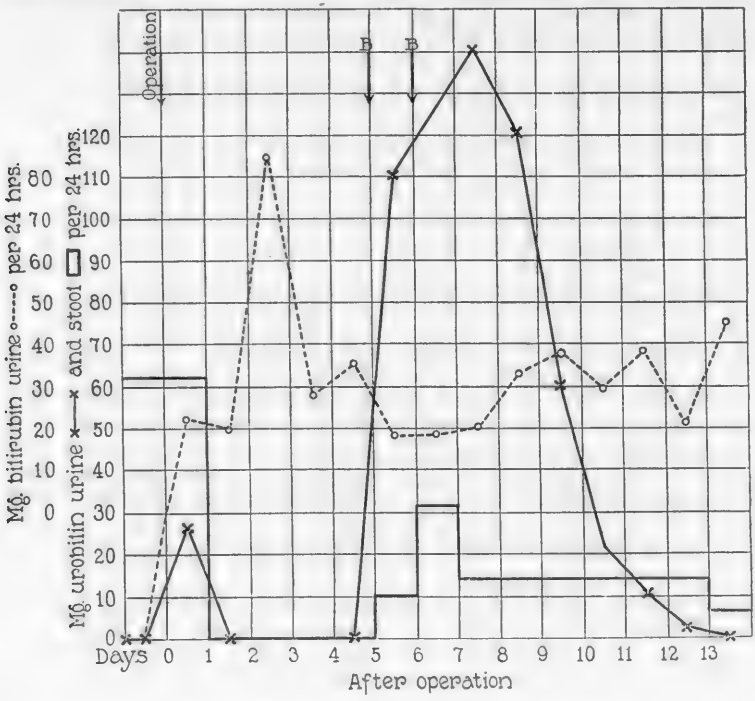
Specimen Protocols.

X. Female dog, weight 11 kilos (see Text-fig. 10).

Under ether anesthesia, intubation of the common duct had been performed. The bile collected afterwards remained sterile, and both stools and bile were urobilin-free. 9 weeks after operation urobilin-free bile was fed daily by stomach tube for a period of 10 days. The amounts given contained bilirubin in approxi-

mately the quantity lost by the intubation. Soon after feedings were begun, urobilin reappeared in the bile and stool; there was, however, no urobilinuria.

Beginning with the 9th day of the feedings, the outlet tube from the liver was clamped for 48 hours. A most striking urobilinuria ensued, 600 mg. of the pig-


Operation
B
B
Mg. bilirubin urine ----- per 24 hrs.
Mg. urobilin urine —×— and stool □ per 24 hrs.
0 10 20 30 40 50 60 70 80
0 10 20 30 40 50 60 70 80 90 100 110 120
Days 0 1 2 3 4 5 6 7 8 9 10 11 12 13
After operation

TEXT-FIG. 12. Marked urobilinuria following the administration of urobilin-free bile to a dog with total biliary obstruction.

At operation the common duct was ligated and cut and the gall bladder removed. Urobilin disappeared from the stool and urine in 3 days, but promptly reappeared in the latter within 18 hours after the administration of urobilin-free bile by gavage. There was no change in the bilirubinuria (see Protocol XII).

ment being excreted through the kidneys in 24 hours. There was only mild bilirubinuria. On release of the obstruction and resumption of bile flow, both pigments disappeared from the urine.

XI. Male dog, weight 18 kilos (see Text-fig. 11).

The common duct of this animal had been intubated under ether, 6 weeks

before. At the time of the experiment, urine, stools, and bile were urobilin-free, and uniform amounts of billirubin were being excreted in the bile from day to day.

A brief anesthetization with chloroform was carried out (50 cc. during 30 minutes). The biliary disturbance that followed was moderate. During the 2nd day after the anesthesia, the 24 hour bilirubin output dropped from 93 to 36 mg. The animal was still urobilin-free, as in other similar experiments already described (Protocols I and II). At this point, bile containing 100 mg. of bilirubin, but no urobilin, was fed by stomach tube, a dose which, as our experience shows, fails to cause urobilinuria in the absence of liver injury. In the present instance the urine voided in the next 24 hours contained 90 mg. of the pigment and the bile 45 mg.

The feeding was not repeated, and the urobilin in the secretions gradually diminished, disappearing wholly in 3 days.

XII. Male dog, weight 11 3/4 kilos (see Text-fig. 12).

Under ether, the common and cystic ducts were cut between ligatures, and the gall bladder evacuated. There followed, as always, transient urobilinuria, but soon the pigment disappeared from both urine and stool (see Protocol VI). On the 6th and 7th postoperative days, after the animal had become urobilin-free, bile which contained none of this pigment was fed by stomach tube, 100 mg. of bilirubin being administered in this way on each day. Large amounts of urobilin were avoided in the urine in the period immediately following, and only after 6 days did the urine become free of it. Small amounts appeared in the stools as well. The bilirubinuria of the persisting biliary obstruction appeared to be uninfluenced by the feedings of bile.

DISCUSSION.

Urobilin and urobilinogen will, of necessity, be considered together in this discussion of their behavior under pathological conditions. For, during our pigment quantitations, the latter is always oxidized into urobilin.[5] In previous papers,[6,7] we have shown that the normal presence of urobilin in the feces and bile depends upon the passage of bilirubin to the intestine, where it is changed to urobilin, and thence reabsorbed in part, to be taken out of circulation again by the liver and partly, at least, secreted into the bile. Complete bile deprivation, as when the common duct is intubated, results in complete disappearance of urobilin from bile and stool. Exclusion of bile from the intestine by direct closure of the common duct leads to similar results.

In the present paper we have shown that these facts hold true even under circumstances of severe liver damage, when unaccompanied by infection. Many workers suppose that the pathologically altered

liver cell can make urobilin, and that this explains the appearance of the pigment in large amounts when the liver is diseased.[4,18,19] Some individuals have doubted the occurrence,[1] but our observations would appear to be the first to prove that it does not take place. On the contrary, the liver loses, when injured, the normal power of taking care of the urobilin resorbed from the intestine, and thus of preventing urobilinuria. We have found that intubated dogs, losing all of their bile, remain urobilin-free after the most severe hepatic injury, as they also do during total biliary obstruction, when there is no passage into the intestines of bilirubin from the jaundiced organism (see Protocols I, II, and VII). Bile samples, also, from dogs with but a part of the liver intubated, and showing urobilin regularly in bile and stool, never contain increased amounts of urobilin as the result of hepatic injury; urobilin behaves in this respect like bilirubin, and in severe cases of injury when "white bile" is being secreted, is absent altogether from this secretion, from intestines, and from urine (see Protocols III and V). Urobilin in the bile, then, in instances of liver damage uncomplicated by infection, would appear to have the same source as in the normal animal[5] and no other. It is not produced by the damaged but sterile liver. Whether it is produced within infected hepatic tissue will be discussed in another communication.

Urobilinuria during liver disease depends upon the formation of urobilin in the intestine, as is revealed in the evidence here reported. In our experiments it was never found even after the most severe hepatic injury in dogs losing the total bile, but appeared promptly after the feeding of an amount of urobilin-free bile far too small to cause urobilinuria in the absence of liver damage (see Protocols X, XI, and XII). In animals losing but a fraction of the bile, urobilinuria occurred promptly after liver damage (see Protocol IV) but disappeared in case there was bile suppression, with result that none reached the intestine (see Protocols III and IV).

The conception of urobilin pathology that accords with the facts observed in our experiments is that the liver, receiving urobilin or urobilinogen by resorption from the intestine into the portal blood

[18] Fischler, F., Physiologie und Pathologie der Leber, Berlin, 1916, 185.
[19] Whipple, G. H., *The Harvey Lectures*, 1921–22, xvii, 103.

under normal circumstances, loses, when diseased, part or all of its ability to dispose of this pigment, which, instead of being removed from the blood, passes on into the general circulation and is excreted by the kidneys. The findings in clinical instances of catarrhal jaundice seem to support the conception outlined which is, of course, not new, but has been weakly supported. Urobilinuria is found only at the beginning and the end of catarrhal jaundice.[1] The transient, early appearance of this pigment parallels our findings in complete obstruction in dogs (see Protocol VI). As the diseased condition resolves, and bilirubin is again discharged into the intestine, urobilin is again formed, absorbed, and, reaching a liver still too damaged to handle it, is passed on and appears in the urine. As the hepatic cells completely recover their ability, the urine again becomes urobilin-free.

The clinical occurrence of urobilin in instances of portal cirrhosis goes to confirm the conception outlined. Urobilinuria may occur in such cases without bilirubinuria, jaundice, or any evidence of excessive hemolysis or of biliary obstruction.[1] The escape of portal blood directly into the general circulation by way of various anastomotic channels is an essential feature of the pathological picture of portal cirrhosis. Thus the urobilin absorbed from the intestinal tract may reach the kidneys before it does the liver, and hence appear in the urine. When there is biliary obstruction or hepatic damage in the absence of a cirrhosis some of the urobilin carried to the liver is passed on and so enters the general circulation, reaching the kidney. The mechanism of the urobilinuria is different; the result is the same.

How does urobilin behave under conditions of local biliary obstruction? It has been shown in this laboratory by McMaster and Rous[16] that in dogs and monkeys over 80 per cent of the liver may be obstructed before bilirubinuria will result, and that in the dog more than 95 per cent of the organ must be occluded before jaundice occurs. We can now say that with urobilin the margin is far narrower. It has been shown in experiments described above that urobilinuria occurs regularly when local obstruction of one-third of the liver is induced, such experiments being easily carried out by clamping the outlet tube draining a small fraction of the total bile, while the rest goes to the intestine (see Protocols VIII and IX). The facts, as observed, cannot be explained by supposing a higher renal threshold

for bilirubin than for urobilin, since in the dog, as already mentioned, bilirubin is, to all intents and purposes, not a threshold substance.

The importance of these relationships lies in their bearing on the interpretation to be accorded urobilinuria in the diagnosis and prognosis of liver disease. Urobilinuria is a valuable finding in that it makes manifest biliary disturbances of mild degree, perhaps not discernible in the urine in any other way. The presence of bile salts might be utilized but too little is known at present concerning its significance. Bilirubinuria occurs only when liver damage or obstruction is considerable. In such cases the finding of urobilin as well, would indicate, in the absence of liver infection, that bile is still reaching the duodenum (see Protocols III and V).

The evidence thus far assembled was obtained from the study of animals with uninfected livers, yielding sterile bile. How will the infection of the liver with urobilin-producing bacteria affect our conclusions? That one may actually produce urobilin *in vitro* by inoculating sterile urobilin-free bile is well known.[20] We have been able to accomplish the same thing *in vivo* by introducing fecal bacteria into the biliary tract of intubated animals. Within 1 or 2 days thereafter the infected secretion is no longer dark, clear, and urobilin-free, but light brown, with a fetid odor, and it contains large amounts of urobilin. The possibility that urobilin formed in this way, in an infected biliary tract, may be resorbed therefrom and excreted in the urine will be taken up in detail in a paper to follow. It constitutes, theoretically at least, an extraintestinal origin of urobilinuria. But it must be clearly pointed out that the phenomenon, if it occurs, is extraneous, having nothing to do with liver function or with urobilin physiology as such, no matter how important it may be clinically.

The experimentally induced formation of urobilin in an infected biliary tract gives a ready explanation of certain discrepancies between our findings and those of others. Fischler[18] always found urobilin in the bile of dogs with open common duct fistulas, and observed it to increase in amount after the administration of certain agents injurious to the liver, such as chloroform, amyl alcohol, and phosphorus. He concluded, therefore, that the diseased liver was able to produce urobilin. Analysis of the findings revealed a number of objections

[20] Kämmerer, H., and Miller, K., *Deutsch. Arch. klin. Med.*, 1923, cxli, 318.

to this conclusion, as Fischler himself pointed out. Our own recent work has given several obvious explanations. Not only does bile given by mouth,—and Fischler's dogs obtained bile by licking their fistula opening,—bring about the appearance of urobilin in the bile, but in fistulous tracts that are infected urobilin arises directly by a change from bilirubin.[6]

Whipple[21] found urobilin regularly in the bile obtained from his open fistula animals during fasting periods, but then only. He believed it to have arisen by the action upon bilirubin of bacteria in the fistula tract. During a fast the bile flow is so sluggish that there would seem to be sufficient time for such bacterial activity to have effect. Very recently, Fischler[22] has described some complicated observations in rabbits, which seem to show that the damaged liver may be able to make urobilin. The liver was injured by the production of "hypoglycemic intoxication," induced by the administration of phlorizin, often with adrenalin, to the fasting animal. Bile was excluded from the intestine by an operative occlusion of the common duct. Urobilinuria was found, especially after the animal had been rescued from the phlorizin intoxication by the use of glucose. Under the conditions, as Fischler describes them, the liver was drastically injured,—for that is a striking effect of sudden biliary obstruction in the rabbit,—while furthermore the jaundice gave opportunity for bilirubin to pass directly into the intestine from the gut wall.[5] Moreover, no mention is made by Fischler of tests to ascertain whether the liver and bile ducts remained sterile. It is well known that bacteria frequently localize in damaged hepatic tissue.

SUMMARY.

A variety of evidence is presented, all of which supports the view that in the uninfected animal the intestinal tract is the only place of origin of urobilin, not merely under normal circumstances, but when there is biliary obstruction. Animals rendered urobilin-free by collection of all of the bile from the intubated common duct remain urobilin-free even after severe hepatic injury.

[21] Whipple, G. H., Robscheit, F. S., and Hooper, C. W., *Am. J. Physiol.*, 1920, liii, 236.

[22] Fischler, F., and Ottensooser, F., *Deutsch. Arch. klin. Med.*, 1925, cxlvi, 305.

In our experiments urobilinuria was never found after liver damage except when bile pigment was present in the intestine. Thus, for example, it appeared during the first days after ligation of the common duct, but disappeared as the stools became acholic. When this had happened a small amount of urobilin-free bile, given by mouth, precipitated a prompt urobilinuria. After obstruction of the duct from one-third of the liver, mild urobilinuria was found, but no bilirubinuria. In animals intubated for the collection of a part of the bile only, while the rest flowed to the duodenum through the ordinary channels, liver injury caused urobilinuria, unless indeed it was so severe as to lead to bile suppression, when almost at once the urobilinuria ceased, though the organism became jaundiced.

The evidence here presented, when taken with that of our previous papers, clearly proves that urobilinuria is an expression of the inability of the liver cells to remove from circulation the urobilin brought by the portal stream, with result that the pigment passes on to kidney and urine. Urobilinuria occurs with a far less degree of liver injury than does bilirubinuria.

Our work has, for the most part, been carried out with animals having uninfected livers and bile passages. But the influence of cholangitis with infection has been briefly discussed in the light of some preliminary observations. The influence of infection on the place of formation of urobilin and on the occurrence of urobilinuria will form the subject of another communication.